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Substantia nigra pars reticulata units in 6-hydroxydopamine-lesioned rats: responses to striatal D2 dopamine receptor stimulation and subthalamic lesions

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Abstract

In order to increase our understanding of Parkinson's disease pathophysiology, we studied the effects of intrastriatally administered selective dopamine receptor agonists on single units from the substantia nigra pars reticulata of 6-hydroxydopamine (6-OHDA)-lesioned rats with or without an additional subthalamic nucleus lesion. Nigral pars reticulata units of 6-OHDA-lesioned rats were classified into two types, showing regular and bursting discharge patterns, respectively ('non-burst' and 'burst' units). Non-burst and burst units showed distinct responses to intrastriatal quinpirole (the former were excited and burst units inhibited). Furthermore, subthalamic nucleus lesions significantly decreased the number of nigral units showing a spontaneous bursting pattern, and reduced the proportion of units that responded to quinpirole. In contrast, subthalamic lesions did not alter the proportion of nigral units that responded to SKF38393, although the lesions changed some response features, e.g. response type and magnitude. Burst analysis showed that quinpirole did not modify the discharge pattern of burst units, whereas SKF38393 produced a shift to regular firing in 62% of the burst units tested. In conjunction, our results support that: (i) the subthalamic nucleus has an important influence on output nuclei firing pattern; (ii) striatal D2 receptors have a strong influence on nigral firing rate, and a less relevant role in controlling firing pattern; (iii) burst and non-burst units differ in their response to selective stimulation of striatal dopamine receptors; (iv) the effects of striatal D2 receptors on nigral units are mainly, though not exclusively, mediated by the subthalamic nucleus; and (v) nigral responses to SKF38393 involve the subthalamic nucleus.

Introduction

According to current pathophysiological models of Parkinson's disease (PD), reduced stimulation of striatal dopamine receptors (DARs) that follows degeneration of dopaminergic neurons results in hyperactivity of basal ganglia output nuclei (DeLong, 1990; Wichmann & DeLong, 1996). Output nuclei neurons are often assumed to be under the control of striatal D1 DARs via the 'direct pathway' (the GABAergic striatonigral/striatoentopeduncular projection) and striatal D2 DARs via the 'indirect pathway' (Gerfen & Young, 1988; Gerfen *et al*., 1990; Wichmann & DeLong, 1996; Levy *et al*., 1997). The indirect pathway comprises the GABAergic (γ-aminobutyric acid) striatopallidal projection, the GABAergic pallido-subthalamic projection, and the glutamatergic subthalamo-nigral/entopeduncular projections. Experimental findings reported by Gerfen *et al*. (1990) suggested an excitatory effect of D1 receptors on striatonigral neurons and an inhibitory action of D2 receptors on striatopallidal neurons. Thus, in PD, the lack of striatal dopamine would lead to an increase of the mean firing rate of output nuclei neurons (Miller & DeLong, 1988; Filion & Tremblay, 1991), as a consequence of two concurrent processes, a reduced activity of the inhibitory direct pathway, and an increased activity of the subthalamic nucleus (STN). Increased STN activity is assumed to result from disinhibition of striatopallidal neurons and the subsequent reduction of the tonic inhibitory influence of the globus pallidus (GP) on the STN (DeLong, 1990; Gerfen *et al*., 1990; but see Chesselet & Delfs, 1996; Levy *et al*., 1997).

This pathophysiological model is supported by experiments revealing compatible neurochemical and metabolic changes in the basal ganglia in animal models of PD (DeLong, 1990; Gerfen *et al*., 1990; Wichmann & DeLong, 1996; Levy *et al*., 1997), and by the striking fact that STN high-frequency stimulation or lesion ameliorates parkinsonian signs in animal models and humans (Bergman *et al*., 1990; Benazzouz *et al*., 1993; Limousin *et al*., 1995). However, electrophysiological studies of single units in the basal ganglia output nuclei provided data that cannot be easily conciliated with the classic basal ganglia model. Recent reports suggested that what is modified in parkinsonism is the neuronal firing pattern rather than the mean firing rate of output nuclei neurons (Murer *et al*., 1997a; Rolfs *et al*., 1997). Briefly, the mean firing rate of substantia nigra pars reticulata (SNpr) units from 6-hydroxydopamine (6-OHDA)-lesioned rats is not significantly different from that of healthy rats. Furthermore, 40% of the SNpr units recorded from lesioned animals discharged action potential bursts, while most units from healthy rats had a regular activity pattern (Murer *et al*., 1997a,b; Rolfs *et al*., 1997). Thus, output nuclei neurons from parkinsonian animals can be classified into two types, 'non-burst units' (which resemble units from healthy animals) and 'burst units' (which are rarely found in animals with an intact nigrostriatal system). It was

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also shown (Burbaud *et al.*, 1995; Murer *et al.*, 1997a) that the proportion of burst units in the SNpr of 6-OHDA-lesioned rats was significantly reduced by STN lesions, a fact that suggested a role for the indirect pathway in the genesis or maintenance of bursting activity.

On the basis of the above revised findings, we hypothesized that SNpr burst and non-burst units would exhibit distinct responses following selective stimulation of striatal D1-class or D2-class DARs. We expected that burst units would shift their pattern to a regular one after D2 DAR stimulation. We further speculated that the effects of striatal D2 DAR stimulation, but not those induced by selective D1 stimulation, would be modified by STN lesions.

Materials and methods

Animals and experimental design

Male adult Sprague–Dawley rats weighing 190–230 g at the time of the first surgical procedure were used for the experiments. They were maintained on a 12 h light : 12 h dark cycle, with food and tap water available *ad libitum*. All the experiments were performed following the NIH's Guide for Care and Use of Laboratory Animals. The experiments were carried out in two groups of animals: (i) rats having a lesion of dopaminergic mesencephalic neurons caused by the toxin 6-OHDA (Ungerstedt, 1971, 6-OHDA rats); and (ii) rats with a 6-OHDA lesion of mesencephalic dopaminergic neurons plus a STN lesion produced by kainic acid (Murer *et al.*, 1997a, 6-OHDA + STN rats). Single units were isolated from the SNpr in order to study their spontaneous activity and their response to intrastriatal administration of selective DAR agonists. The localization of intrastriatal injection sites, nigral recording sites and the extent of STN lesions was determined from Nissl-stained sections. The effectiveness of the 6-OHDA treatment was evaluated both by behavioural and immunohistochemical methods. For a detailed description of the methods see Murer *et al.* (1997a).

Unilateral 6-OHDA lesions

The rats were anaesthetized with pentobarbital (50 mg/kg i.p.) and fixed to a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The neurotoxin 6-OHDA hydrobromide (Sigma, St Louis, MO, USA, 8 μg free base in 4 μL of 0.1% ascorbic acid) was injected at the medial forebrain bundle according to the following stereotaxic coordinates, referred to the interaural line: A, 3.6; L, 1.5; and H, –2.7 (Pellegrino *et al.*, 1979). The solution was administered over an 8-min period, through a stainless steel cannula (0.3 mm O.D.), connected to a microsyringe (10 μL; Hamilton, Reno, NV, USA) driven by a microdrive unit. The extent of the 6-OHDA-induced lesion was estimated 12–15 days after surgery by measuring the rats' contralateral turning response to apomorphine (0.1 mg/kg s.c.). Only those animals showing more than 100 full contralateral net turns in 1 h were selected for the experiments.

Subthalamic nucleus kainic acid lesions

STN lesions were performed following an already described procedure (Murer *et al.*, 1995; 1997a). Briefly, a solution containing kainic acid [Sigma, 0.5 μg in 0.2 μL of phosphate-buffered saline (PBS), pH 7.4] was injected at the following stereotaxic coordinates, referred to the interaural line: A, 4.3; L, 2.2; and H, –2.7 (Pellegrino *et al.*, 1979). The solution was delivered over a 90-s period, through a stainless steel cannula (0.3 mm O.D.), connected to a Hamilton microsyringe (10 μL) driven by a microdrive unit. Upon recovering from anaesthesia the rats received diazepam (10 mg/kg i.p.) to prevent distant brain damage and to avoid seizures (Ben-Ari *et al.*, 1979). In some animals a similar procedure was accomplished, but without kainic acid in the solution (6-OHDA + sham STN rats). Because data obtained from 6-OHDA + sham STN rats and 6-OHDA rats were similar, they were pooled for analysis.

In vivo electrophysiological single-unit recording

Extracellular single-unit recording was performed in rats anaesthetized with urethane (1.2 g/kg i.p.) and secured to a stereotaxic frame (David Kopf). Body temperature was maintained at 37 ± 0.5 °C with a feedback-controlled heating pad, and additional urethane was administrated as needed. Two glass microelectrodes with tip diameters of 2–5 μm (1–10 MΩ), filled with 1% Pontamine Sky Blue in 2 M NaCl, were placed in the SNpr at the following coordinates, referred to the interaural line (Pellegrino *et al.*, 1979): A, 2.6; L, 1.9–2.5; and H, –2.5 to –3.0. The signals were passed through high-impedance amplifiers and filters (bandwidth 300–3000 Hz; P511, Grass Instruments, Quincy, MA, USA), displayed on an oscilloscope (Tektronix 565, Beaverton, OR, USA), monitored with an audio amplifier, and recorded on videotape (DR-890, NeuroData, Delaware Water Gap, PA, USA) for off-line analysis. The two microelectrodes were hydraulically advanced through the SNpr until single units could be isolated. Identification of SNpr single units was achieved following previously described electrophysiological criteria (Murer *et al.*, 1997a,b).

The isolated units were monitored for at least 10 min to assure the stability of their firing rate, firing pattern and spike morphology, and then 5–10 min of spontaneous activity was recorded. Then, a microinjection of the selective D1-class DAR agonist SKF38393 (RBI, Natick, MA, USA, 10 nmol/0.5 μL), or of the selective D2-class agonist quinpirole (RBI, 10 nmol/0.5 μL) was performed in the striatum [coordinates from the interaural line (Pellegrino *et al.*, 1979): A, 8.0; L, 2.7–3.3; and H, +1.0 to +1.5]. This dose was selected in order to facilitate comparison with previous work (Murer *et al.*, 1997a,b). The drugs were dissolved in 0.01% ascorbic acid solution or PBS, and administered through a stainless steel needle (0.3 mm O.D.) connected to a Hamilton microsyringe (10 μL) driven by a Baltimore microdrive. This procedure demanded less than 2 min, and the needle was left in place until the end of recording. In several experiments we were able to record simultaneously two different SNpr units for at least 50 min after the injection. At the end of the experiment the position of the electrode tips was marked by an iontophoretic deposit of Pontamine Sky Blue and the rat was transcardially perfused with cold saline followed by 4% paraformaldehyde in PBS.

To determine the pharmacological specificity of the effects of quinpirole and SKF38393, we tried to prevent their effects on units of the SNpr by systemic administration of selective DAR antagonists. Once a pair of SNpr units was isolated and a control period of spontaneous activity recorded, we administered either the selective D1-class antagonist SCH23390 (RBI) or the D2-class antagonist eticlopride (RBI, 0.5–1 mg/kg, s.c.). Thirty minutes later an intrastriatal injection of SKF38393 or quinpirole was accomplished as described above.

All experiments were carried out 4–8 weeks after the behavioural test in all rats. The dose of apomorphine used for behavioural testing (0.1 mg/kg, s.c.), and the time interval between the behavioural test and the recording session were chosen following the work of Morelli *et al.* (1989), who found that the effect of a priming dose of apomorphine (0.1 mg/kg, s.c.) reached a peak after 3 days and had almost disappeared by the 10th day postinjection. By using a small dose of apomorphine in a single test, and delaying the recording session by 4–8 weeks, we tried to minimize the effect of priming on

our results. In 6-OHDA + STN rats, STN lesions were performed 5–8 days before the recording procedure.

Histology and immunohistochemistry

After perfusion, the rats' brains were removed and postfixed for 2 h before being incubated in 15% sucrose in PBS for 24 h. The brains were frozen, stored for 2–3 days at –20 °C, and then, 30-µm-thick sections were cut in a freezing microtome. The free-floating sections were incubated for 30 min in a PBS solution containing 2% bovine serum albumin (Sigma) and 0.2% Triton X-100 (Sigma), washed and incubated at 4 °C for 48 h with monoclonal antibodies directed against tyrosine hydroxylase (TH, 1 : 1000, Boehringer Mannheim, Indianapolis, IN, USA). After washing, the sections were incubated for 2 h with biotinylated antimouse IgG (1 : 200, Amersham, Arlington Heights, IL, USA), washed again and incubated for 2 h in an avidin–biotin–peroxidase complex (1 : 100, Vectastain Elite ABC kit, Vector, Burlingame, CA, USA). The antigen–antibody complexes were visualized using 3,3′-diaminobenzidine (Sigma) as chromogen.

Data analysis

The stored signal was converted to square wave pulses with the aid of a window discriminator (Mentor N750, Minneapolis, MI, USA) and led to an A to D converter and microcomputer. For each unit we have calculated: (i) the mean discharge frequency (integrated over 30-s epochs); (ii) the frequency distribution of the interspike intervals (ISIs) and their coefficients of variation (CV); and (iii) the autocorrelograms. Autocorrelograms and ISI histograms were computed from at least 500–1000 intervals obtained from representative segments of signal, both at control and postinjection periods. Visual analysis of digital raster displays complemented the study of ISI histograms and autocorrelograms, allowing the classification of units according to their firing patterns of activity (Murer *et al*., 1997a). In addition, we implemented an algorithm described by Legéndy & Salcman (1985) to evaluate the 'degree of burstiness' of the recorded units. This method was used to obtain an objective classification of SNpr units on the basis of their spontaneous activity, and allowed us to study the effect of the drugs on their discharge pattern. Briefly, bursts were defined as at least three consecutive ISIs with a duration shorter than half that of the mean ISI of the signal. For each detected burst, the algorithm stores the number of spikes it contains and its surprise. The surprise of a burst is a measure of how improbable it is to find, by chance, a similar sequence of ISIs in a neuron with a random (Poisson) spike discharge pattern. For the classification of units on the basis of their spontaneous activity, we studied a segment of signal encompassing 3000 spikes. Units showing more than 5% of the spikes within bursts were classified as burst units. To analyse the effects of drugs on burstiness, the length of signal used to calculate the surprise had to be adjusted in order not to exceed the duration of the drug-induced response. For some units we had to limit the analysis to a segment encompassing 1000 spikes during the postinjection period, and to recalculate burst parameters for a length of signal encompassing a similar number of spikes during the control period.

Units showing at least six consecutive 30-s epochs with a firing rate deviated more than 15% from the mean frequency of a 5-min preinjection (control) period were considered as responsive. This ± 15% range was selected after studying the firing rate changes of 15 units injected with the vehicle alone, during a 40-min postinjection period (see also Murer *et al*., 1997a,b). Only those units that recovered their control firing rate after changes induced by the pharmacological treatments were considered for the study of the effects of DAR agonists.

The Fisher's exact probability test (FET), Student's *t*-test, and two-way ANOVAs were required to determine the statistical significance of differences between groups, pharmacological treatments and types of units.

Results

Data were obtained from 35 rats with 6-OHDA lesions, nine rats with 6-OHDA lesions and sham STN lesions, and 28 rats with 6-OHDA lesions and STN lesions. Another 21 rats with 6-OHDA-induced lesions were used for the study of the pharmacological selectivity of the intrastriatal drug treatments. The total number of units available for the analysis of SNpr spontaneous activity was higher than that useful for the study of drug-induced effects, as many SNpr units were lost shortly before or after the intrastriatal injection of DAR agonists.

Histology and immunohistochemistry

All 6-OHDA-lesioned rats used throughout this study showed more than 100 full contralateral net turns during 1 h after administration of apomorphine (0.1 mg/kg s.c., mean ± SEM = 258.6 ± 17.9). Immunohistochemistry for TH revealed an almost complete absence of TH-immunoreactive neurons in the substantia nigra pars compacta and ventral tegmental area ipsilateral to the 6-OHDA injection, and of TH-immunoreactive fibres in the lesioned forebrain side (Fig. 1). The STN was almost completely destroyed by kainic acid and the zona incerta suffered a slight damage, as reported (Murer *et al*., 1995; 1997a, Fig. 2). Almost all recording sites were located within the lateral half of the SNpr, between stereotaxic planes A2.4 and A2.8, while striatal injections sites were placed between A8.0 and A8.2 (Pellegrino *et al*., 1979, Fig. 1).

Spontaneous activity of substantia nigra pars reticulata units

Single units isolated from 6-OHDA rats ($n = 129$) showed two different firing patterns (Murer *et al*., 1997a). One kind of unit showed a regular or slightly irregular pattern of activity, at least two peaks in autocorrelograms, and symmetric ISI histograms (resembling units from the SNpr of healthy rats), and will collectively be called 'non-burst units' (Murer *et al*., 1997a, Fig. 3). The remaining units showed a bursting activity pattern, with very asymmetric or bimodal ISI histograms, and flat autocorrelograms with a single initial peak (Murer *et al*., 1997a, Fig. 3, Table 1). Their long-duration and high-frequency action potential bursts were separated by periods of low-frequency tonic discharge or complete absence of firing. Thirty-six per cent of the recorded units were classified as burst units following the algorithm described by Legéndy & Salcman (1985) (Table 1). Burst units fired action potentials at a significantly slower mean rate than non-burst ones, and showed significantly higher CVs of their ISIs (Table 1). Burst and non-burst units had steady spontaneous firing patterns. In several cases their spontaneous activity was recorded for more than 1 h, and we never observed any spontaneous shift in firing pattern from one category to the other.

Forty-three units were recorded from the SNpr of 6-OHDA + STN rats. Among them, 19% belonged to the burst type, a proportion significantly lower than that found in 6-OHDA rats (FET, $P < 0.0001$, Table 1). Two-way ANOVAs with group (6-OHDA versus 6-OHDA + STN rats) and firing pattern (non-burst versus burst units) as factors, did not show statistically significant differences between groups for firing rate, nor for the CV of the ISIs, of the different kinds of units (Table 1). Furthermore, no difference was found in burst measurements (number of bursts, of spikes per burst, and surprise)

between the burst units of 6-OHDA rats and those of 6-OHDA + STN rats. Thus, the main effect of STN lesions was a reduction of the proportion of burst units in the SNpr.

Effects of SKF38393

Intrastriatal administration of SKF38393 (10 nmol/0.5 μL) produced responses in 52% ($n = 13$ out of 25) of the SNpr units recorded from 6-OHDA rats (54% were excited and 46% inhibited, Table 2 and Fig. 4). Burst units seemed to respond with higher probability (66.7%, $n = 8$ out of 12) than non-bursts units (38.5%, $n = 5$ out of 13), but the difference did not reach statistical significance (FET, $P = 0.24$). The proportion of excitatory and inhibitory responses induced by the drug was not different between burst and non-burst units (Table 2). We have already reported (Murer *et al.*, 1997b) that 23% ($n = 3$ out of 13) of the SNpr units recorded responded to intrastriatal SKF38393 in healthy rats, a proportion not significantly different from that obtained in 6-OHDA rats (FET, $P = 0.16$).

STN lesions did not modify the proportion of SNpr units that responded to intrastriatal SKF38393 in 6-OHDA rats (53.3%, $n = 8$ out of 15, FET, $P = 1$, Table 2), but all the responses were excitatory

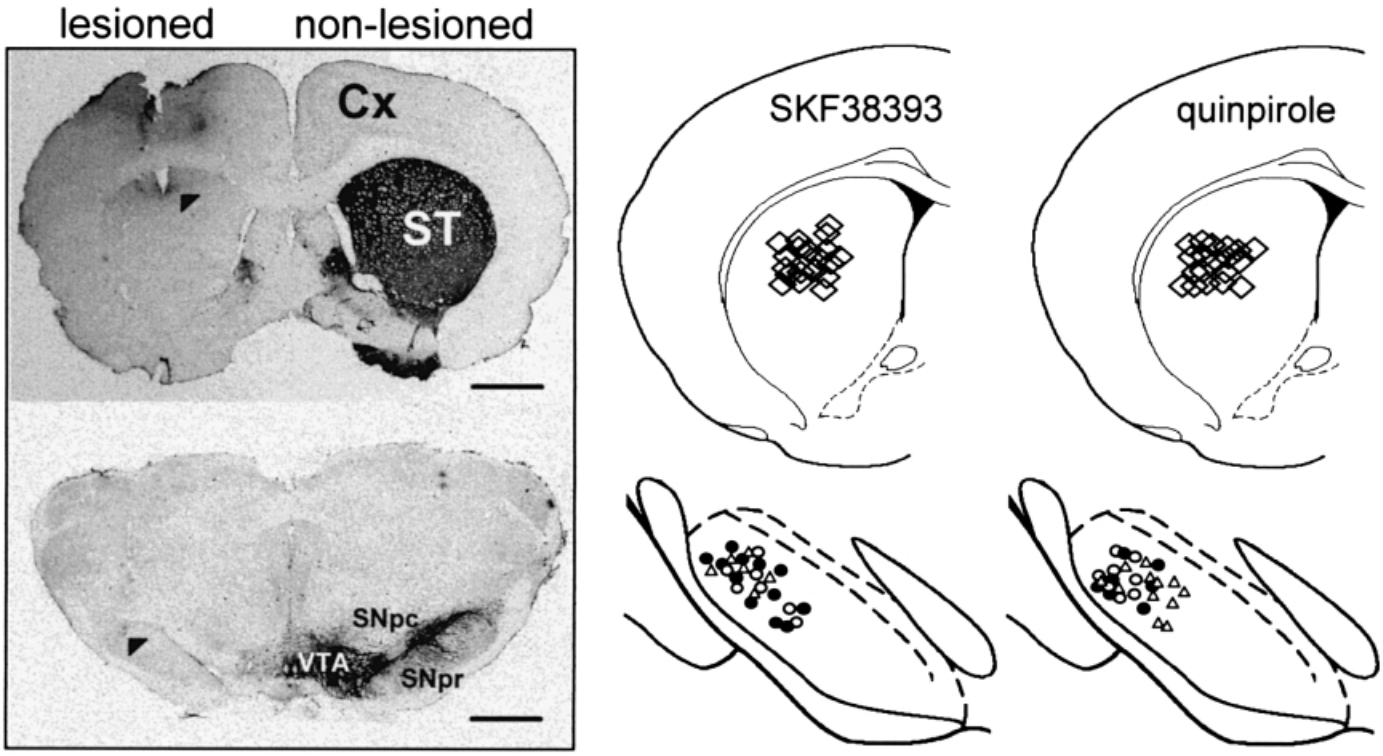

FIG. 1. Left, low-power bright-field images of sections stained with antibodies directed against tyrosine hydroxylase, showing the extent of a representative 6-OHDA-induced lesion. The arrowheads indicate the location of the intrastriatal injection site and nigral recording site. Scale bars, above, 1.5 mm; below, 1 mm. Cx, cortex; ST, striatum; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; VTA, ventral tegmental area. Right, schematic drawings of coronal sections of the rat brain showing the injections sites (striatum) and recordings sites (substantia nigra) of 6-OHDA-lesioned rats treated with SKF38393 or quinpirole. Filled circles, no response; empty circles, excitation; empty triangles, inhibition.

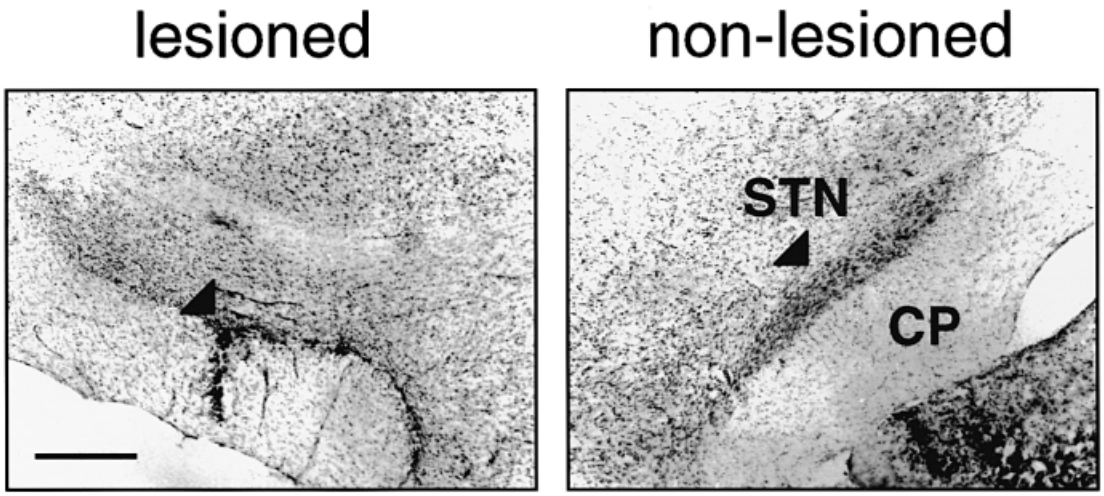

FIG. 2. Nissl-stained sections demonstrated gliosis and an almost complete destruction of neurons in the subthalamic nucleus after kainic acid injections. CP, cerebral peduncle; STN, subthalamic nucleus. Scale bar, 0.4 mm.

in 6-OHDA + STN rats (FET, $P = 0.04$). Furthermore, those SNpr units responding to SKF38393 in 6-OHDA + STN rats showed smaller changes in firing rate than units excited by SKF38393 in 6-OHDA rats (Student's *t*-test, $P = 0.007$, Table 2). Thus, in 6-OHDA rats, STN lesions modified the type of response of SNpr units to intrastriatal SKF38393 and reduced the SKF38393-induced mean firing rate change, without altering the proportion of responsive units.

Effects of quinpirole

Intrastriatal quinpirole (10 nmol/0.5 µL) induced responses in 74% ($n = 20$ out of 27) of SNpr units from 6-OHDA-lesioned rats (40% were excited and 60% inhibited, Table 2 and Fig. 5). Bursts units responded with higher probability (91%, $n = 10$ out of 11) than non-burst units (62.5%, $n = 10$ out of 16), but the difference did not reach statistical significance (FET, $P = 0.18$). The proportion of excitatory and inhibitory responses was, respectively, 80% and 20% for non-bursts units. In striking contrast, all responsive burst units were inhibited by intrastriatal quinpirole (FET, $P = 0.0007$, Table 3 and Fig. 5). We have recently reported (Murer *et al.*, 1997b) that only 17% (two out of 12) of the SNpr units tested responded to intrastriatal quinpirole in healthy rats. This proportion is significantly lower than that reported now for 6-OHDA rats (FET, $P = 0.0014$).

Finally, we found that the proportion of SNpr units responsive to intrastriatal quinpirole was significantly lower (33.3%, $n = 5$ out of 15, Table 2) in 6-OHDA + STN rats than in 6-OHDA rats (FET, $P = 0.02$).

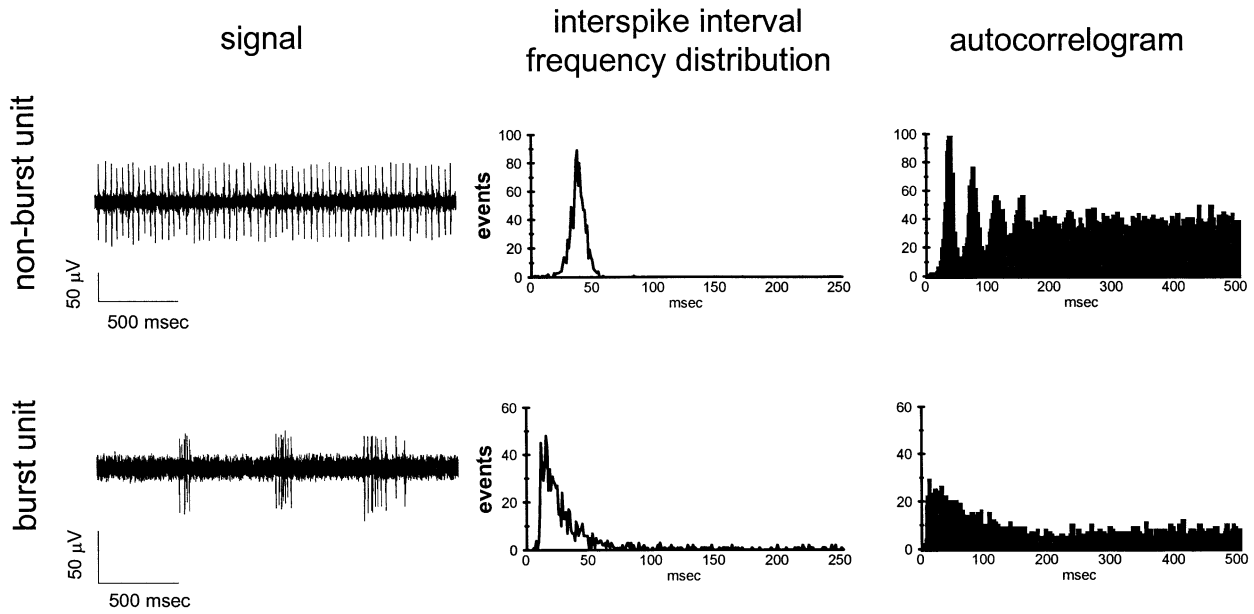

FIG. 3. Single units from the substantia nigra pars reticulata of 6-OHDA-lesioned rats could be classified into two types on the basis of their pattern of activity. Non-burst units showed a more or less regular discharge pattern, a symmetric interspike interval frequency distribution and several peaks in autocorrelograms. Burst units showed strongly asymmetric interspike interval frequency distributions and flat autocorrelograms with a single initial peak.

TABLE 1. Spontaneous activity of substantia nigra pars reticulata single units recorded from 6-OHDA-lesioned rats with or without a kainic acid lesion of the subthalamic nucleus

	Units (*n*)	Units* (%)	Firing rate†	CV of the ISIs§	Number of bursts	Spikes per burst (*n*)	Surprise
6-OHDA							
Non-burst	82	63.6	19.2 ± 0.94	0.31 ± 0.01			
Burst	47	36.4	14.7 ± 0.94‡	0.79 ± 0.07¶	86.9 ± 8.4	5.84 ± 0.32	2.76 ± 0.25
Total	129	100	17.6 ± 0.71	0.48 ± 0.03			
6-OHDA + STN							
Non-burst	35	81.4	23.5 ± 1.37	0.27 ± 0.01			
Burst	8	18.6	17.1 ± 4.07‡	0.88 ± 0.16¶	85.4 ± 28.6	5.43 ± 0.58	2.02 ± 0.23
Total	43	100	22.3 ± 1.37	0.39 ± 0.05			

Data are the mean ± SEM of *n* neurons per group. The units were classified following the algorithm described by Legéndy & Salcman (1985) (see Materials and methods). *Fisher's exact probability test demonstrated significantly different proportions of burst and non-burst units between 6-OHDA and 6-OHDA + STN rats, $P < 0.0001$. †Two-way ANOVA did not show significant differences between the firing rates of units obtained from 6-OHDA and 6-OHDA + STN rats ($F_{1,168} = 3.69$, $P = 0.056$), but burst units showed a significantly lower mean firing rate than non-burst ones (‡$F_{1,168} = 9.64$, $P < 0.002$). The interaction between groups and patterns of discharge was not statistically significant ($F_{1,168} = 0.28$, $P = 0.599$). §Two-way ANOVA did not show significant differences between the CVs of the ISIs of units obtained from 6-OHDA and 6-OHDA + STN rats ($F_{1,168} = 0.22$, $P = 0.641$), but burst units showed a significantly higher CV than non-burst ones (¶$F_{1,168} = 76.49$, $P < 0.000001$). The interaction between groups and patterns of discharge was not statistically significant ($F_{1,168} = 1.02$, $P = 0.310$). No significant difference was found between burst parameters of burst units from 6-OHDA and 6-OHDA + STN rats (Student's *t*-test).

Effects of dopamine receptor agonists on the firing pattern of substantia nigra pars reticulata units

Among the non-burst units studied, just one shifted its firing pattern to the burst type after intrastriatal administration of SKF38393, and two after quinpirole. For burst units, only one of those that responded to quinpirole changed its firing pattern to the non-burst type. The remaining nine burst units which were inhibited by quinpirole continued to display bursting activity during the response (Fig. 5 and Table 3). In fact, these neurons showed a significant increase in the number of bursts ($P = 0.013$, Student's *t*-test for paired data) without changes in other burst features (Table 3). In contrast, after intrastriatal SKF38393, five of the eight responsive burst units shifted their firing pattern to the non-burst type (Fig. 4 and Table 3, $P = 0.023$, FET, versus burst units challenged with quinpirole). The different effects of SKF38393 and quinpirole on firing pattern seemed to be related to the firing rate changes induced by the drugs. Thus, four out of the five burst units showing a regular pattern after intrastriatal SKF38393 displayed an excitatory response (all the units excited by SKF38393 shifted their patterns to the non-burst type).

TABLE 2. Subthalamic nucleus lesions differentially alter the response of substantia nigra pars reticulata units to intrastriatal administration of D1-class- and D2-class-selective agonists in 6-OHDA-lesioned rats

	6-OHDA rats	6-OHDA + STN rats
Number of responding units [responding/total (%)]		
SKF38393	13/25 (52.0%)	8/15 (53.3%)
Quinpirole	20/27 (74.1%)	5/15 (33.3%)*
Response features		
SKF38393		
Inhibited/total (%)	6/13 (46.1%)	0/8 (0%)
Mean firing rate change (% of control)	–61 ± 11	–
Excited/total (%)	7/13 (53.8%)	8/8 (100%)†
Mean firing rate change (% of control)	66 ± 7	38 ± 6‡
Quinpirole		
Inhibited/total (%)	12/20 (60%)	3/5 (60%)
Mean firing rate change (% of control)	–47 ± 6	–66 ± 7
Excited/total (%)	8/20 (40%)	2/5 (40%)
Mean firing rate change (% of control)	37 ± 7	72§

STN lesions reduced the probability of responding of SNpr units to intrastriatal quinpirole without changing the probability of responding to SKF38393. *$P = 0.02$, Fisher's exact probability test, versus 6-OHDA rats. STN lesions changed the type of response of SNpr units to intrastriatal SKF38393. †$P = 0.04$, Fisher's exact probability test, versus 6-OHDA rats. STN lesions reduced the magnitude of the excitatory responses induced by intrastriatal SKF38393. ‡$P = 0.007$ versus 6-OHDA rats, Student's *t*-test. §SEM could not be calculated for two units.

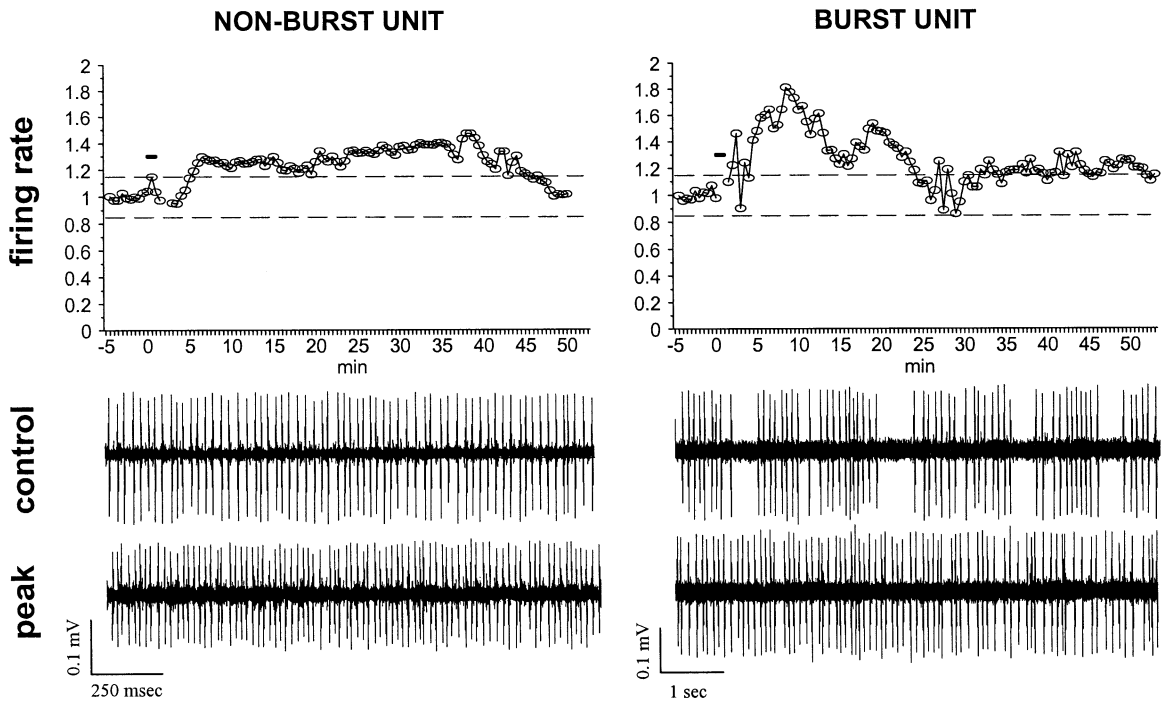

FIG. 4. Effects of intrastriatal SKF38393 (10 nmol/0.5 μL) on the activity of single units recorded from the substantia nigra pars reticulata of 6-OHDA-lesioned rats. The time-course of the response and signal samples obtained from the control period (control) and at the peak of the response (peak) are shown for a non-burst and burst unit. Note that the excitatory response of the burst unit is accompanied by a regularization of the unit's firing pattern.

Effects of selective dopamine receptor antagonists on SKF38393- and quinpirole-induced responses

When injected 30 min after systemic administration of the selective D2-class antagonist eticlopride (0.5–1 mg/kg s.c.), quinpirole was unable to alter the firing rate of any of the six units tested (Table 4). In contrast, most units challenged with intrastriatal SKF38393 after systemic eticlopride responded (57%). On the other hand, after a systemic treatment with the selective D1-class antagonist SCH23390 (0.5–1 mg/kg s.c.), any of the eight units tested with intrastriatal SKF38393 responded (Table 4), whereas most cells injected with intrastriatal quinpirole changed their firing rate beyond the ± 15% control interval (71%). Further evidence on the pharmacological specificity of the effects of intrastriatal SKF38393 and quinpirole has already been provided (Murer *et al*., 1997a,b), by demonstrating: (i) a synergistic effect of their intrastriatal coadministration on SNpr units of healthy rats; and (ii) that the effects of the intrastriatal coadministration of SKF38393 and quinpirole matched those of intrastriatal apomorphine on SNpr units from healthy rats.

Discussion

The main findings of this study can be summarized as follows: (i) in agreement with previous reports (Burbaud *et al*., 1995; Murer *et al*., 1997a), we found that STN lesions reduced the proportion of burst units in the SNpr of 6-OHDA-lesioned rats; (ii) intrastriatal quinpirole produced firing rate changes in ~75% of the SNpr units tested, but it could not reverse the firing pattern of nigral burst units into a more regular one; (iii) burst and non-burst units differ in their

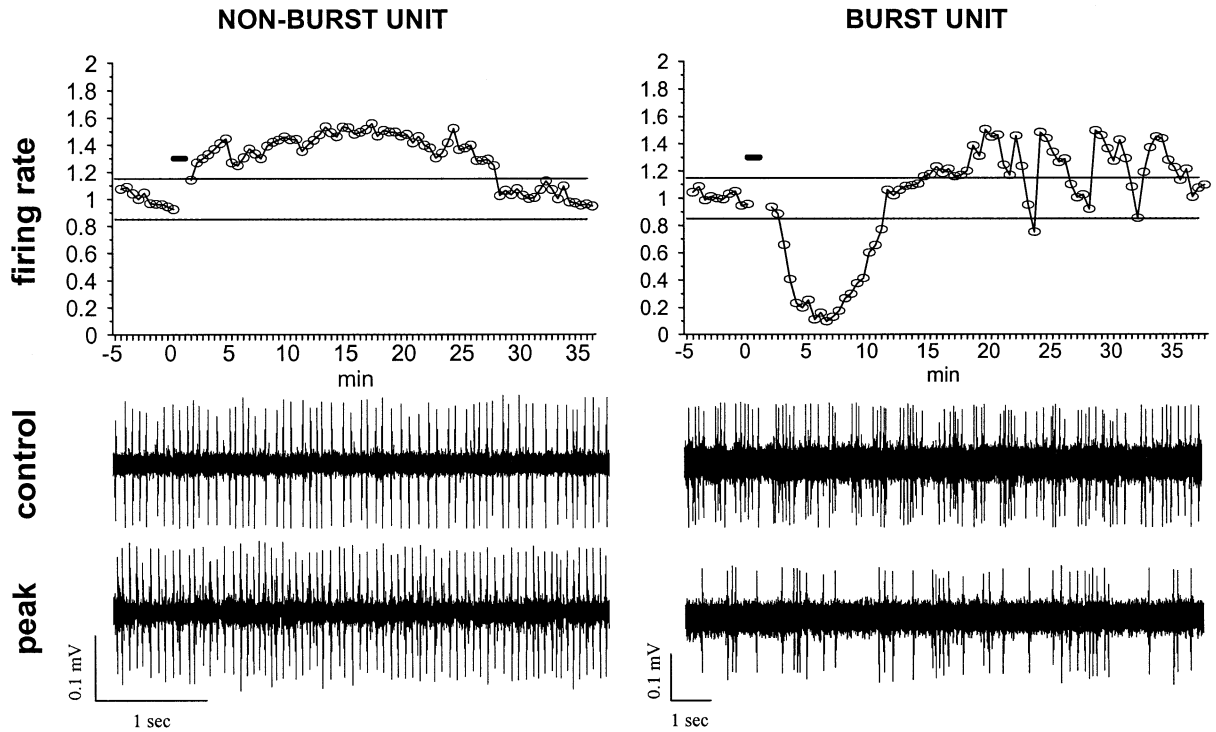

FIG. 5. Effects of intrastriatal quinpirole (10 nmol/0.5 µL) on the activity of single units recorded from the substantia nigra pars reticulata of 6-OHDA-lesioned rats. The time-course of the response and signal samples obtained from the control period (control) and at the peak of the response (peak) are shown for a non-burst and burst unit. Note that a bursting pattern can still be observed in the burst unit during the inhibitory response.

TABLE 3. Burst and non-burst units showed distinct responses after intrastriatal administration of selective dopamine receptor agonists

	Number of units responding/total		Still showing bursts after injection	Changes relative to the preinjection control period (%)		
	Excited	Inhibited	(Bursting/responsive)	Number of bursts	Spikes per burst	Surprise
SKF38393						
Non-burst	3/13	2/13				
Burst	4/12	4/12	3/8	–1.4 ± 25.9	+13.8 ± 20.1	–7.4 ± 13.8
Quinpirole						
Non-burst	8/16	2/16				
Burst	0/11	10/11*†	9/10‡	+36.3 ± 18.3§	–1.1 ± 15.0	–1.4 ± 13.8

Burst and non-burst units showed distinct responses after intrastriatal quinpirole. *$P = 0.0007$, Fisher's exact probability test, versus non-burst units challenged with quinpirole. Burst units showed distinct responses to intrastriatal D1-class and D2-class dopamine receptor agonists. †$P = 0.023$, Fisher's exact probability test, versus burst units challenged with SKF38393. Most burst units showing drug-induced responses shifted their pattern to the non-burst type after intrastriatal SKF38393, while continued bursting after quinpirole. It should be noted that the three units which still displayed bursts after intrastriatal SKF38393 showed an inhibitory response. ‡$P = 0.04$, Fisher's exact probability test, versus burst units challenged with SKF38393. In fact, burst units exhibited significantly more bursts after intrastriatal quinpirole. §$P = 0.013$, Student's *t*-test for paired data.

TABLE 4. Pharmacological specificity of the effects of selective dopamine receptor agonists on single units of the substantia nigra pars reticulata

Treatment	Number of responsive units	
SKF38393	13/25	(52%)
SKF38393 + SCH23390	0/8	(0%)*†
SKF38393 + eticlopride	4/7	(57%)
Quinpirole	20/27	(74%)
Quinpirole + SCH23390	5/7	(71%)
Quinpirole + eticlopride	0/6	(0%)‡§

*$P = 0.012$ versus SKF38393 alone, Fisher's exact probability test. †$P = 0.026$ versus SKF38393 + eticlopride, Fisher's exact probability test. ‡$P = 0.001$ versus quinpirole alone, Fisher's exact probability test. §$P = 0.021$ versus quinpirole + SCH23390, Fisher's exact probability test.

response to intrastriatal administration of selective DAR agonists; (iv) STN lesions significantly reduced the proportion of SNpr units responsive to stimulation of striatal D2 DARs, although ~30% of the units tested still responded to intrastriatal quinpirole in animals with STN lesions; and (v) STN lesions had conspicuous effects on SNpr responses to intrastriatal SKF38393.

The experiments were performed in urethane-anaesthetized animals. It is well known that anaesthetics can influence the firing rate and discharge pattern of neurons, and their responses to pharmacological treatments. Reports on the spontaneous activity of basal ganglia output nuclei neurons from awake parkinsonian monkeys and patients with PD suggest, however, that bursting activity is a prominent feature of units in both the internal segment of the GP and the SNpr (Filion & Tremblay, 1991; Hutchinson *et al*., 1997; Wichmann *et al*., 1999). In particular, a recent study by Wichmann *et al*. (1999) showing increased bursting activity in SNpr units from awake methyl-phenyl-tetrahydropyridine-lesioned primates, strongly supports our previous findings obtained from urethane-anaesthetized rats (Murer *et al*., 1997a). Several findings further suggest that the pharmacological responses of SNpr units to DAR agonists are not substantially modified by urethane anaesthesia. Thus, Weick & Walters (1987) found that SKF38393 and quinpirole (administered through a tail vein) produced mainly inhibitory responses on SNpr units recorded from paralysed non-anaesthetized 6-OHDA-lesioned rats, but also excitation or no response in a number of neurons. In addition, results reported by Hutchinson *et al*. (1997) on the effects of subcutaneous apomorphine injections on output nuclei neurons recorded from awake patients with PD were similar to those reported by us after intrastriatal apomorphine injections in urethane-anaesthetized 6-OHDA-lesioned rats (Murer *et al*., 1997a).

Spontaneous activity of substantia nigra pars reticulata units in 6-OHDA-lesioned rats

Several recent reports (Miller & DeLong, 1988; Filion & Tremblay, 1991; Burbaud *et al*., 1995; Hutchinson *et al*., 1997; Murer *et al*., 1997a,b; Rolfs *et al*., 1997; Wichmann *et al*., 1999) stressed that one prominent feature of parkinsonism in animals and humans is the presence of a large number of neurons with a bursting discharge pattern in the basal ganglia output nuclei. After 6-OHDA lesions, most SNpr units (non-burst) displayed a regular or slightly irregular firing pattern, resembling that of neurons from healthy rats, while the rest (36%) showed a dramatically different mode of discharge, with action potential burst separated by periods of regular low-frequency firing or complete absence of discharge (Burbaud *et al*., 1995; Murer *et al*., 1997a; Rolfs *et al*., 1997). After STN lesions, the two populations of SNpr units could still be recognized (non-burst and burst), showing firing rates, ISI histograms, autocorrelogram profiles and bursting parameters, which did not differ from those of SNpr units from 6-OHDA-lesioned rats. Nevertheless, the STN seems to be involved in the generation of the abnormal bursting pattern, as its lesion significantly reduced the proportion of burst units in the SNpr of 6-OHDA-lesioned rats. This conclusion is further supported by a recent report by Plenz & Kitai (1999) showing that, in organotypic cultures lacking dopaminergic neurons, the STN and GP display synchronized oscillatory burst discharge.

It is usually assumed that the clinical manifestations of parkinsonism result from an increased firing rate of basal ganglia output nuclei neurons (see Introduction). If that were the case, one would expect STN lesions to reduce the mean firing rate of SNpr units from 6-OHDA-lesioned rats. In our sample, however, STN lesions increased (although non-significantly) the mean firing rate of SNpr units. We have reported a similar result previously (Murer *et al*., 1997a). Thus, we can hypothesize that the behavioural improvement which follows STN lesions in animal models of PD is related to changes in the spontaneous firing pattern of output nuclei neurons, or to modifications in the response of output nuclei neurons to other afferent pathways.

Distinct effects of striatal D1 and D2 dopamine receptors on nigral units and the role of the subthalamic nucleus

The present report shows that burst and non-burst units can be distinguished on the basis of their response to striatal D2-class DAR stimulation. Whereas non-burst units were commonly excited by intrastriatal quinpirole, all burst units were inhibited. This fact suggests that SNpr units receive heterogeneous inputs from striatal neurons that express D2-class DARs. The GP, which is assumed to rely information from D2-expressing striatofugal neurons to the SNpr (see Introduction), is also constituted by an heterogeneous population of neurons, which can be separated in categories by their electrophysiological properties *in vitro* (Nambu & Llinas, 1994) and *in vivo* (Kita & Kitai, 1994b; Kelland *et al*., 1995), by their differential response to DAR agonists *in vivo* (Carlson *et al*., 1990; Kelland *et al*., 1995), and by their projection fields and content of parvalbumin (Kita & Kitai, 1994a,b). It seems plausible that striatal D2 DARs affect SNpr unit activity both through the direct projection from the GP to the SNpr (Smith & Bolam, 1989; 1991) and the GP–STN–SNpr circuit (DeLong, 1990; Wichmann & DeLong, 1996). This hypothesis is consistent with the fact that ~30% of the SNpr units tested still responded to intrastriatal quinpirole in rats with STN lesions. It could even be that the effects of striatal D2 DARs on SNpr units were conveyed in part by the striatonigral projection. This possibility is suggested by recent reports demonstrating: (i) synaptic contacts between striatonigral and striatopallidal neurons in the striatum (Aronin *et al*., 1986; Bolam & Izzo, 1988; Yung *et al*., 1996); (ii) that activation of striatal D2 DARs potentiate the effects of D1-class agonists on striatonigral neurons (Gerfen *et al*., 1995); and (iii) colocalization of D1-class and D2-class DARs on subpopulations of striatofugal neurons (Surmeier *et al*., 1996). There is another experimental finding which suggests divergent roles of striatal D2 DARs and the STN in the control of SNpr single unit activity. STN lesions produced a slight (non-significant) increase in the mean firing rate of SNpr units but had a dramatic effect on their firing pattern, reducing the proportion of burst units in 6-OHDA-lesioned rats. In contrast, intrastriatal quinpirole produced a significant decrease in the mean firing rate of SNpr burst units but could not revert their firing pattern into a more regular one. On the other hand, the fact that STN lesions significantly reduced the proportion of SNpr units responsive to intrastriatal quinpirole supports the classical concept about the STN functioning as a link between striatal D2 DARs and output

nuclei neurons. This conclusion is in good agreement with that of Anderson *et al*. (1992), who showed that STN lesions reduced contralateral turning behaviour induced by systemic quinpirole in 6-OHDA-lesioned rats. Thus, it seems that striatal D2 DARs affect SNpr neural activity through circuits which either do or do not involve the STN.

The lesion of the STN had subtle effects on SNpr responses evoked by intrastriatal SKF38393. Firstly, inhibitory responses to intrastriatal SKF38393 could not be found in 6-OHDA-lesioned rats after STN lesions; second, STN lesions reduced the magnitude of excitatory responses to intrastriatal SKF38393. Likewise, other recent reports suggested the existence of interactions between the direct and indirect pathways. Thus, D1-class agonists can modify the expression of molecules and the electrophysiological activity of neurons of the indirect pathway (Carlson *et al*., 1990; Ruskin & Marshall, 1995; Kreiss *et al*., 1996, 1997). In fact, systemic administration of D1-class agonists has more dramatic effects on STN neuronal activity than administration of D2-class agonists (Kreiss *et al*., 1996, 1997). Both the colocalization of D1 and D2 receptors on striatofugal neurons and the synaptic contacts between striatonigral and striatopallidal neurons mentioned above can explain the fact that STN lesions modified SNpr responses to intrastriatal SKF38393. Furthermore, it is known that most striatonigral neurons send axon collaterals to the GP, at least in the rat (Kawaguchi *et al*., 1990). In conclusion, the complexity of the effects of STN lesions on SNpr responses induced by selective stimulation of striatal D1 and D2 DARs is not consistent with the existence of two completely segregated direct (D1-influenced) and indirect (D2-influenced) pathways linking the striatum with the output nuclei.

Bursting activity and the pathophysiology of parkinsonism

If bursting activity is related to the clinical manifestations of PD, one could expect that treatments possessing clinical efficacy would lead to a regularization of output nuclei neuronal activity. We have reported recently (Murer *et al*., 1997a) that intrastriatal apomorphine produced strong inhibitory responses in SNpr burst units of 6-OHDA-lesioned rats, but was unable to revert the abnormal firing pattern of most burst units. Similarly, intrastriatal quinpirole could not revert the abnormal firing pattern of burst units despite its ability to reduce their mean firing rate. In contrast, SKF38393 had excitatory effects on several SNpr burst units and simultaneously regularized their firing pattern. It is commonly accepted that D1-class agonists are less effective than D2-class or non-selective DAR agonists for the treatment of PD (Poewe, 1998). Our results suggest that the clinical efficacy of DAR agonists is not related to their ability to regularize output nuclei neuronal firing pattern. This contention is consistent with reports suggesting that in subjects with PD, acute apomorphine administration produced a clinical benefit that correlated with a reduction of output nuclei neuronal firing rate (Hutchinson *et al*., 1997). On the other hand, STN lesions, which reverted parkinsonian signs in primates, had a dramatic effect on SNpr firing pattern but did not significantly change SNpr mean firing rate. It could be that dopamine agonists and STN lesions lead to clinical benefit through different mechanisms.

In summary, the pathophysiological significance of bursting activity remains obscure. It could be that bursting activity results from a very severe dopamine depletion, thus representing a feature of late stage parkinsonism. If this is the case, the inability of DAR agonists to regularize output nuclei firing pattern could explain why DAR agonists lose efficacy as PD progresses (Poewe, 1998). This speculation is consistent with the observation that therapeutic strategies aimed at modifying STN function (which could probably lead to changes in output nuclei firing pattern) are effective in advanced PD (Limousin *et al*., 1995). Further work is necessary to confirm this hypothesis.

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Abbreviations

6-OHDA, 6-hydroxydopamine; CV, coefficient of variation; DAR, dopamine receptor; FET, Fisher's exact probability test; GABA, γ-aminobutyric acid; GP, globus pallidus; ISI, interspike interval; PBS, phosphate-buffered saline; PD, Parkinson's disease; SNpr, substantia nigra pars reticulata; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

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