

The Electrophysiological Effect of
Subthalamic Lesion and Dopamine D₁ and
D₂ Receptor Agonists in Basal Ganglia
Nuclei in Parkinsonian Rat Model

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Nuclei in Parkinsonian Rat Model

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Abstract

The Electrophysiological Effect of Subthalamic Lesion and Dopamine D₁ and D₂ Receptor Agonists in Basal Ganglia Nuclei in Parkinsonian Rat Model

The loss of dopamine from the striatal dopamine receptor causes subthalamic nucleus (STN) neuron hyperactivity, and leads to the hyperactivity of the output nuclei of the basal ganglia. Ways of reducing STN hyperactivity have been studied since they this was found to reverse the Parkinsonian motor symptoms. Subthalamic nucleus lesions decrease the output of the STN, and normalize the temporary disorder in the motor controlling system in basal ganglia. Another approach taken to reduce the activity of the STN involves the stimulation of striatal dopamine D₁ and D₂ receptor using dopaminergic agonists, which leads indirectly to a decrease in STN neuronal activity.

In this study, SKF38393 (a D₁ receptor agonist) and Quinpirole (a D₂ receptor agonist), reported to decrease the activity of the inhibitory pallido-subthalamic pathway, were consecutively injected intrastriatally. The substantia nigra pars reticula (SNpr) and the ventrolateral thalamic nucleus (VL) were microrecorded to ascertain the activity of the basal ganglia output

structure. The effects of STN lesioning by using kainic acid (1 mg/0.5 ml, total 1 μ l) on the neuronal activities of SNpr and VL were investigated by monitoring firing rates and firing patterns. Firing patterns was treated as a combination of a regular pattern and a bursting pattern. In the SNc lesioned rats, an SKF38393 injection decreased the firing rate of SNpr (26 ± 2.3 spikes/s 19 ± 2.9 spikes/s), but increased the firing rate of VL (4 ± 2.2 spikes/s 7 ± 0.8 spikes/s). The firing rate of SNpr was decreased (29 ± 1.9 spikes/s 16 ± 0.4 spikes/s), but the firing rate of VL (5 ± 0.7 spikes/s 13 ± 3.3 spikes/s) increased, by injecting Quinpirole. The proportion of burst neurons, however, was unaffected. On the other hand, STN lesioning decreased both the firing rate and the proportion of burst neurons.

The effect of SKF38393 or Quinpirole injection on the firing rate of SNpr and VL was investigated in SNc + STN lesioned rats. Compared to the result in SNc lesioned rats, the firing rate of SNpr decreased to a lesser extent and the firing rate of VL was increased to a lesser extent. These results demonstrate that lesioning of the STN decreases the hyperactivity of the SNpr and the proportion of burst neurons on total neurons, but dopamine receptor agonists such as SKF38393 and Quinpirole did not change the firing pattern. It was also found that STN could mediate the action of SKF38393

and Quinpirole.

Key Words: 6-Hydroxydopamine, Parkinson's disease, subthalamic nucleus, basal ganglia, kainic acid, dopamine agonist

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. Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder induced by a progressing deficit of dopamine cells in the pars compacta of the substantia nigra (SNc), leading to dopamine depletion in the striatum.¹ This dopamine reduction is responsible for the imbalance in the activities of the direct and indirect pathways relating the striatum to the basal ganglia output structures, the pars reticulata of the substantia nigra (SNpr) and the internal part of globus pallidus (Gpi).^{2,3} According to current models of basal ganglia circuitry, changes in the activity of both direct and indirect inputs from the striatum to the internal pallidum and the substantia nigra pars reticulata are

significant in hypokinetic movement disorders, such as akinesia in Parkinson's disease.⁴ The direct pathway comprises the GABAergic striatopallidal projection, the GABAergic pallido-subthalamic projection, and the glutamatergic subthalamo-nigral/entopeduncular projections. In the indirect pathway, the subthalamic nucleus (STN) exerts excitatory (glutamatergic) control on the output structures of the basal ganglia.⁵ According to this model, the STN acts primarily as a relay between the external pallidum and the internal pallidum/substantia nigra in the indirect pathway.⁴ In accord with this hypothesis, events thought to decrease subthalamic neuronal output have been found to reverse the behavioral effects of dopamine reduction in rats,^{6,7} primates,^{8,9,10} and humans.^{8,9,11} Dopamine plays a critical role in regulating the striatal neuronal activity via the D₁ and D₂ subtypes of the dopamine receptors. The D₁ receptors are present on the striatal output neurons projecting to the SNpr and the entopeduncular nucleus (EPN), while the D₂ receptors are present on the neurons projecting to the globus pallidus (GP) in rats.¹² According to recent pathophysiological models of PD, the reduced stimulation of the striatal dopamine receptors that follows degeneration of dopaminergic neurons results in hyperactivity of the basal ganglia output nuclei.¹³ Therefore, dopaminergic agonists are predicted to exert a therapeutic effect at the level of the subthalamus by indirectly reducing

STN neuronal activity via stimulating striatal dopamine D₂ receptors.¹⁴

The motor nuclei of the thalamus [ventromedial nucleus (VM) and VL in the rat] function between the basal ganglia motor circuit and the cortex.^{2,3} The ventrolateral nucleus (VL) is known to receive direct GABAergic projections from the output structures of the basal ganglia.² According to the widely accepted functional organization of the basal ganglia, the change in the firing activity of output structures of the system is supposed to induce an increase in the tonic inhibitory change exerted by these structures on the activity of motor thalamic nuclei, resulting in the deactivation of motor cortical areas.^{1,2}

The effects of STN lesioning, SKF38393 (a D₁ agonist) and Quinpirole (a D₂ agonist) were compared in a Parkinsonian rat model in this study. To investigate these effects the firing rates and firing patterns of SNpr neurons were analyzed. The firing rates and patterns of VL neurons were also analyzed, since SNpr neurons are supposed to affect VL neurons.^{1,2,3} To investigate Parkinsonism pathophysiology the effects of SKF38393 and Quinpirole were also determined on the STN lesioned Parkinsonian rat model.

. Materials and Methods

1. Surgical procedures for the medial forebrain bundle (MFB) and for the STN lesion

Male adult Sprague-Dawley rats at weighting 200-250 g were used for the first surgical procedure. Rats were divided into 5 groups: (i) control group, 7 rats without lesions (including both non-lesioned and sham-lesioned animals); (ii) a 6-OHDA lesioned group (n = 7) that had a lesion of the dopaminergic mesencephalic neurons induced by a toxin 6-OHDA; () a 6-OHDA + STN lesioned group, 7 rats with a 6-OHDA lesion of mesencephalic dopaminergic neurons plus a STN lesion, induced by kainic acid; and () a 6-OHDA + STN sham group, 7 rats treated with saline instead of kainic acid in the STN. Five animals per group were housed in a temperature-controlled room on a 12hr.-light/12hr.-dark schedule with free access to food and water. Rats were anesthetized with a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg) and rompun (4 mg/kg) and mounted in a stereotaxic apparatus. A neurotoxin 6-OHDA hydrobromide (Sigma, St Louis, MO, 8 µg free base in 4 µl of 0.2% ascorbic acid) was injected into the medial forebrain bundle according to the following stereotaxic coordinates: AP -4.4 mm, ML 1.2 mm relative to bregma, and DV -7.5 mm from the dura. The injection was made at

a rate of 0.5 μ l/min using a cannula, and was controlled using a Hamilton microsyringe. The connection between the cannula and the microsyringe was composed of polyethylene tubing. To prevent the noradrenergic neurons being destroyed, desipramine (12.5 mg/kg, i.p.) was administered 30 min prior to the 6-OHDA infusion.

STN lesioning was achieved by injecting 1 μ g of kainic acid (Sigma, St Louis, MO) dissolved in 0.5 μ l of saline into the right STN (coordinates: AP -3.8 mm, ML 2.5 mm relative to bregma, and DV -8.0 mm from the dura) at the rate of 0.25 μ l/min. In the group of rats with paired lesions (6-OHDA + STN lesion), STN lesioning was performed 3 weeks after 6-OHDA lesioning. Sham lesioning was performed by using the same protocols as used for the paired lesions, but saline was injected instead of kainic acid.

2. Extracellular microrecordings

Extracellular, single unit recordings were produced from rats anesthetized with urethane (1.3 mg/kg i.p.). A glass microelectrode (impedance 7-10 Mohm) filled with 2.5% Pontamine Sky Blue in 0.5 M sodium acetate buffer (pH 7.6) was used to produce the single recordings. Microelectrodes were stereotaxically guided through a drilled skull burr hole to the target

coordinates (SNpr: AP -5.3 mm, ML 2.4 mm relative to bregma, and DV 7.5-8.0 mm from the dura; VL: AP -2.12. mm, ML 1.6 mm relative to bregma, and DV 5.5-6.5 mm from the dura). Electrical signals were amplified using a DAM80 preamplifier (WPI, UK) in bridge mode, displayed on a storage oscilloscope and monitored with an audio amplifier. Single unit activity was isolated with a window discriminator, and firing rate data were collected on a computer equipped with Spike 2 software (version 2.18, Cambridge Electronic Design, UK). Visual inspection of digital neuronal activity and raster displays were useful complements to the computer based analysis of the discharge patterns of these units. The isolated units were monitored for at least 10 min to ensure the stability of, their firing rate, firing pattern and spike morphology, and then 5-10 min of spontaneous activity was recorded. The selective D₁-class dopamine agonist SKF38393 (Sigma, St Louis, MO, 10 nmol/0.5 µl), or of the selective D₂-class agonist Quinpirole (Sigma, St Louis, MO, 10 µmol/0.5 µl) was injected in the striatum [coordinates AP -0.8 mm, ML 3.0 mm relative to bregma, and DV 1.3 mm from the dura]. This dose was selected in order to allow comparisons with previous work. The drugs were dissolved in PBS, and administered through a stainless steel needle (0.3 mm O.D.) connected to a Hamilton microsyringe (10 µl). This procedure required less than 2 min, and the needle was left in place until the

end of the recording. At the end of the experiment the positions of the electrode tips were marked by an iontophoretic deposit of Pontamine Sky Blue, and the rat was transcardially perfused with cold saline followed by 4% paraformaldehyde in PBS. At the end of the recording, the location of the tip of the recording microelectrode was marked, at $-15\ \mu\text{A}$ for 20-30 min, by an iontophoretic deposit of Pontamine Sky Blue. After the recordings had been made animals were deeply anaesthetized, brains were perfused and removed, and later sectioned for histological confirmation of the recording site. The stored signal was converted to square wave pulses with the aid of a window discriminator (WPI, UK) and a personal computer. The mean firing rate, the mean interspike interval (ISI), autocorrelogram and discharge pattern were investigated for each neuron. The ISIs allowed an evaluation of the neurons degree of burst frequency, following an algorithm described by Hutchison et al (1997, 1998).^{5,16} Bursting cells had a degree of burstiness score of more than 10, and were calculated from the reciprocal of the modal interval divided by the mean firing rate.

3. Histology and immunohistochemistry

After the extracellular single unit recording, neurons were identified by their stereotaxic location and by the histological location of the electrode tip

after iontophoresis with Pontamine Sky Blue from the recording electrode (-18 μ A for 20 min). Rats were anesthetized and transcardially perfused with 125 ml of normal saline followed by 250 ml of ice-cold 4% paraformaldehyde. Brains were removed, postfixed for 10 hours, and transferred to 30% sucrose until equilibrated. 20 μ m sections were cut frozen and then immunoreacted with a primary, polyclonal antibody against rat TH (Pel-freeze, Rogers, AK) at a dilution of 1:750, and then with a biotinylated goat anti-rabbit IgG (Vector Labs, Burlingame, CA) secondary antibody. The signal was amplified using avidin and biotinylated horseradish peroxidase using the Elite ABC Vectastain Kit (Vector, Burlingame, CA). 3,3'-Diaminobenzidine tetrachloride dehydrate was used as a chromogen and cobalt chloride/nickel ammonium was used to intensify color changes. This immunostaining allowed us to determine the extent of dopaminergic cell degeneration. Only rats with a total loss of TH immunoreactivity were used for the electrophysiological analysis.

The STN lesions and the localization of the recorded basal ganglia nuclei were studied in 20 μ m sections stained with cresyl violet.

4. Data analysis

Statistical analysis was performed with the SPSS version 9.0 statistical

software package (SPSS Inc., Chicago, IL). Comparisons of the firing rates from different rats in each group were performed using analysis of variance (ANOVA). Results showing significant differences between groups were compared using Kruskal-Wallis one-way ANOVA and then the Mann-Whitney *U*-test. Statistical significance was accepted when p was < 0.05 .

. Results

1. Histological findings of rat PD models

The extent and location of the lesions induced by the 6-OHDA were confirmed by assessing the loss of TH-immunoreactive cells and fibers in the substantia nigra pars compacta (SNc) and striatum in a rat Parkinsonian model with 6-OHDA (Fig. 1A). In addition, the ventral tegmental area (VTA) was also lesioned on the same side in most of the rats. The STN lesions were also evaluated after conducting the experiments and they revealed local gliosis at the level of the STN (Fig. 1B). Those rats in which the STN lesion extended to the nearby basal ganglia nuclei or missed the STN altogether were excluded from the data analysis. The localizations of the recorded SNpr and VL sites were confirmed by cresyl violet staining (Fig. 1C)

2. Effects of STN lesions on firing rate and firing patterns

In each group, the mean firing rates, and the total number of cells recorded are shown in table 1. The number of cells recorded per track was similar for each group. The firing patterns in the SNpr were classified into a regular non-bursting pattern and a bursting pattern (Fig. 2). In normal unlesioned rats

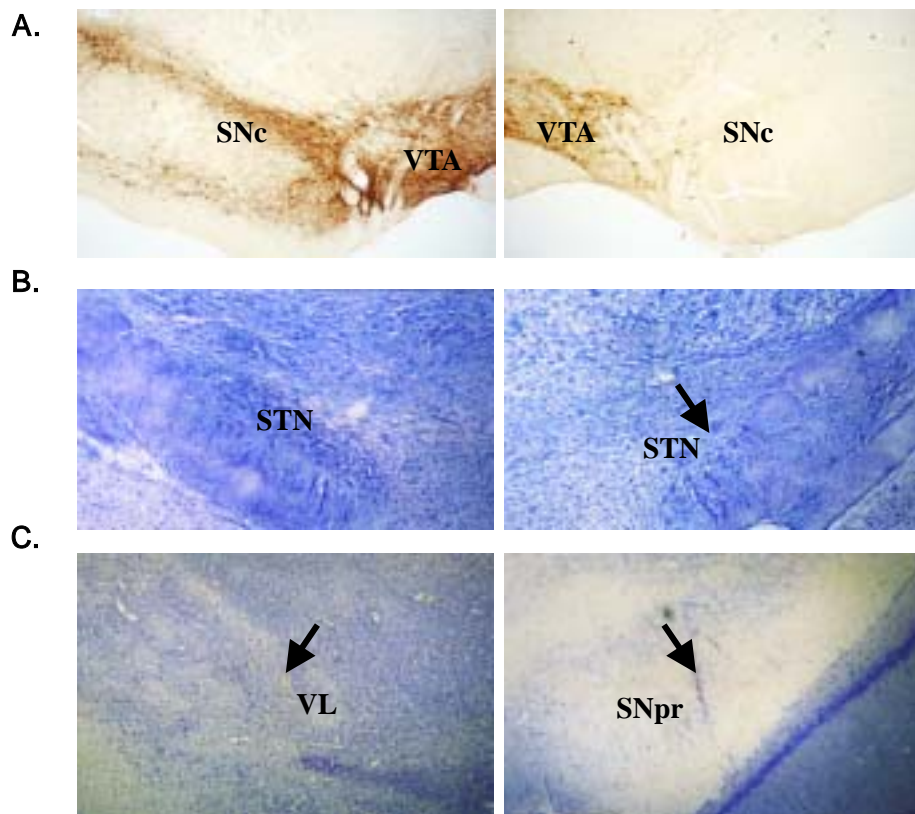


Figure 1. (A) Immunohistochemistry of tyrosine hydroxylase (TH) showing the total degeneration of dopamine fibers in the striatum, and dopamine cell bodies in the SNc on the 6-OHDA injected side (right) compared to the normal side (left). (B) Cresyl violet-stained sections illustrating a unilateral kainic acid lesion in the subthalamic nucleus. Arrow indicates the location of the lesion. (C) Photomicrograph showing the Pontamine Sky Blue mark corresponding to a neuron recorded at the end of a track in the ventrolateral nucleus (left) and pars reticula of substantia nigra (right). Magnification, $\times 40$ SNpc: substantia nigra pars reticula, STN: subthalamic nucleus, VTA: ventral tegmental area, VL: ventrolateral thalamic nucleus, SNpr: substantia nigra pars reticula

($n = 35$), the mean firing rates of neurons in the SNpr were 20 ± 9 spikes/s (table 1). Compared with the normal control rats, PD rat models with 6-OHDA exhibited significantly increased mean firing rates in the SNpr (28 ± 1.5 spikes/s) ($p < 0.05$). Following STN lesioning in the PD rats, the mean firing rate in the SNpr was reduced versus that of PD rats (21 ± 1.8 vs. 28 ± 1.5 spikes/s, respectively) ($p < 0.05$). No statistically significant difference was observed between the mean firing rates of sham STN lesioned and STN lesioned PD rats (28 ± 2.0 spikes/s) ($p > 0.05$). Regular neurons represent

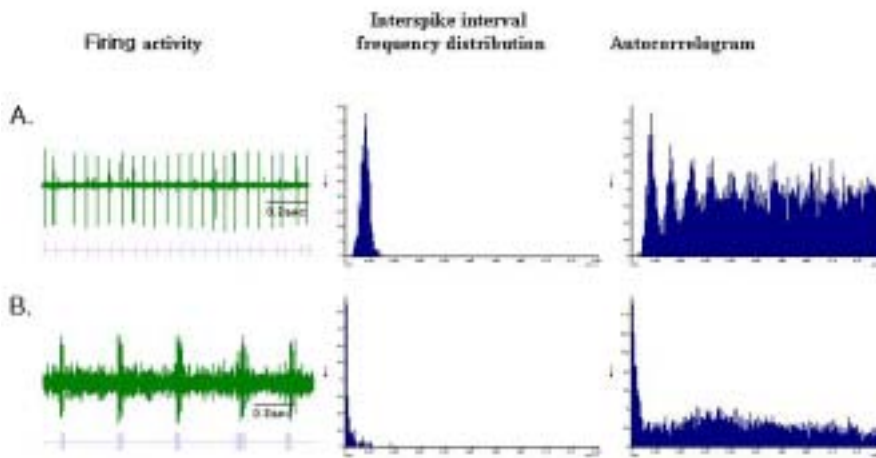


Figure 2. SNpr discharge pattern recorded in 6-OHDA-lesioned rats. Left: Neuronal activity, each dot corresponds to a spike (Scale bar 1.4 s) raster display Middle: ISI histograms Right: autocorrelogram
A. regular non-bursting pattern B. Bursting pattern

76% of the neurons in normal rats, and burst neurons 24% (Fig. 3). In 6-OHDA lesioned rats, the number of burst neurons increased (24% → 35%). STN lesions in the 6-OHDA lesioned rats increased the percentage of regular neurons to 87% in the group of STN lesioned rats versus the 76% of normal rats. In intact rats, VL unit basal firing rates ranged from 4 to 15 spikes/s, with a mean \pm SEM of 8 ± 1.5 spikes/s. In rats with 6-OHDA lesions, the firing rates ranged from 8 ± 1.5 spikes/s to 5 ± 0.6 spikes/s (Table 2), and the proportion of burst neurons decreased from 91% to 82% (Fig. 4).

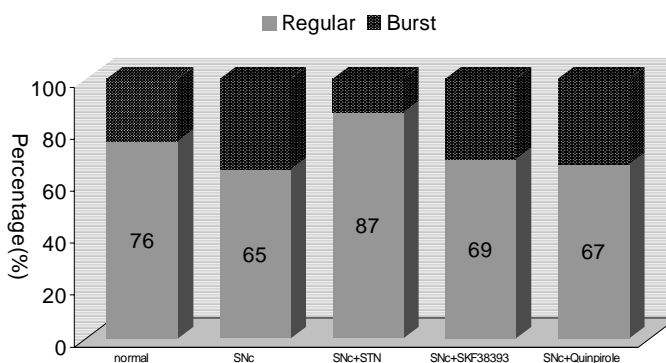


Figure 3. The effects of STN lesioning, D₁ agonists SKF38393 and D₂ agonist Quinpirole on the firing pattern of SNpr neurons. The proportion of burst neurons in the SNpr of 6-OHDA lesioned rats was significantly reduced by STN lesions. SKF38393 and Quinpirole induced a slight reduction in the proportion of burst neurons.

Table 1. Spontaneous activity of SNpr single units recorded from 6-OHDA lesioned rats with a kainic acid lesion of the STN and intrastriatal selective D₁, D₂ agonist microinjection.

	Normal			SNF30000			Quinpirole		
	neurons(n)	mean	Interpike	neurons(n)	mean	Interpike	neurons(n)	mean	Interpike
		Firing rate(Hz)	Interval(sec)		Firing rate(Hz)	Interval(sec)		Firing rate(Hz)	Interval(sec)
Control	42	8±1.5	0.024±0.0071	12	8±2.1	0.013±0.0079	17	6±0.9	0.008±0.0029
PD	36	5±0.6	0.023±0.0057	14	7±0.8	0.025±0.0094	28	11±2.1	0.005±0.0019
PD+STN	20	11±1.8	0.008±0.0023	18	13±1.8	0.017±0.0041	17	9±1.4	0.018±0.0049
PD+STN+ham	19	5±1.5	0.020±0.0047	12	8±1.8	0.047±0.0108	11	12±3	0.009±0.0033

The values means ± SEM. * $p < 0.05$ in comparison with values from normal animals

3. Effects of SKF38393 on firing rates and firing patterns

Effects of the D₁ agonist, SKF38393 applied by intrastriatal injection, on firing rate of SNpr units is showed in table 1. The administration of SKF38393 decreased SNpr neuronal firing rates in lesioned rats (from 28 ± 1.5 to 21 ± 2.7 spikes/s, $n = 25$), whereas the SKF38393 did not significantly alter the mean firing rate of SNpr neurons in the intact rat (21 ± 1.6 vs. the control at 20 ± 1.9 spikes/s, $n = 16$). However, SKF38393 did not alter the mean neuronal firing rate in the SNpr neurons of SNc + STN lesioned rats (20 ± 2.3 vs. 21 ± 1.8 spikes/s, respectively, $n = 18$).

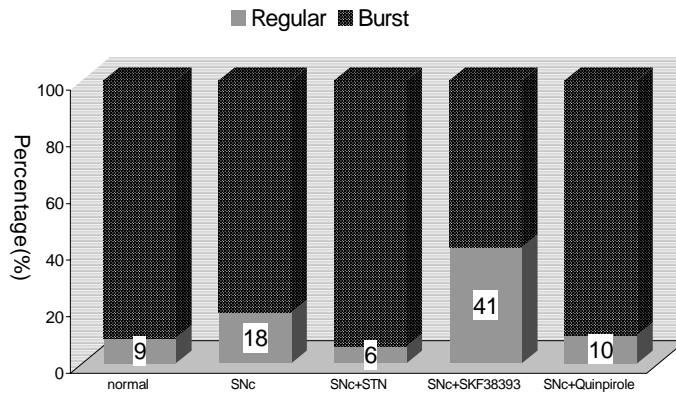


Figure 4. The effects of STN lesioning, D₁ agonists SKF38393 and D₂ agonist Quinpirole on the firing pattern in VL neurons. The proportion of burst neurons in the SNpr of 6-OHDA lesioned rats was reduced. The SKF38393 reduced the proportion of burst neurons.

Table 2. Spontaneous activity of VL units recorded from 6-OHDA lesioned rats with a kainic acid lesion of the STN and intrastratial selective D₁, D₂ agonist microinjection of the striatum

	Normal			SEF3050			Damphetamine		
	neurons (n)	mean firing rate(Hz)	Inter spike interval(sec)	neurons (n)	mean firing rate(Hz)	Inter spike interval(sec)	neurons (n)	mean firing rate(Hz)	Inter spike interval(sec)
Control	95	28±1.9	0.047±0.0071	16	21±1.8	0.093±0.0060	12	28±2.4	0.028±0.0045
PD	47	28±1.5	0.029±0.0036	25	21±2.7	0.045±0.0074	24	16±1.3	0.041±0.0079
PD+STN	27	21±1.8	0.037±0.0049	18	20±2.3	0.061±0.0082	16	24±3.5	0.038±0.0062
PD+STN+ham	21	28±2.0	0.032±0.0055	14	20±4.2	0.053±0.0150	11	18±1.1	0.028±0.0063

The values means ± SEM. * $p < 0.05$ in comparison with values from normal animals

In normal rats, SKF38393 did not induce a change in the firing pattern. In rats with 6-OHDA lesions of the SNc, SKF38393 induced a slight decrease in the percentage of burst neurons (35% → 31%).

The effects of D₁ agonist injection on VL neurons are showed in table 2. In normal rats, the firing rates of the VL neurons in these treatment groups were not significantly different. In 6-OHDA lesioned rats, the administration of SKF38393 increased the firing rates of VL neurons (5 ± 0.6 spikes/s → 7 ± 0.8 spikes/s). In terms of the firing pattern, SKF38393 caused a reduction in the percentage of burst neurons in 6-OHDA lesioned rats (94% → 59%).

4. Effects of Quinpirole on the firing rate and firing patterns

The excitatory effect of dopamine on STN neuronal activity appeared to be largely mimicked by the dopamine D₂-like receptor agonist. Quinpirole increased the mean firing rate of SNpr neurons in normal rats from 20 ± 1.9 to 28 ± 2.4 spikes/s ($p < 0.01$). However, in neurons prepared from 6-OHDA lesioned rats, Quinpirole decreased the spontaneous firing rate from 28 ± 1.5 to 16 ± 1.3 spikes/s ($p < 0.01$). The administration of Quinpirole did not alter the proportion of burst neurons in the SNpr, as shown in figure 3. (whereas STN lesioning significantly altered proportion of burst neurons in the lesioned rats).

The dopamine D₂ receptor agonist Quinpirole increased the neuronal firing rates (from 11 ± 2.1 to 5 ± 0.6 spikes/s), in the VL of lesioned rats, as shown table 2. However, in terms of the firing pattern no proportional change in burst neurons was observed (Fig. 4).

. Discussion

1. Firing rate

We observed hyperactivity in the SNpr of 6-OHDA lesioned rats, as previously reported.^{17,18} In Parkinson's disease, progressive deficit of dopamine cells in the substantia nigra pars compacta leads to impaired information processing in the basal ganglia.^{2,4,8} In particular, it is thought that Parkinsonian pathophysiology results from the over-inhibition of the thalamocortical pathway resulting from the increased activity of basal ganglia output structures, the internal globus pallidus and the substantia nigra pars reticulata. The improved activity of these output structures in the dopamine-depleted state may be due, partly, to an increase in excitatory drive from the STN.²¹ The hyperactivity of the STN is based on the hypothesis that the loss of dopamine in the striatum causes a reduction in the activity of the inhibitory GABAergic pallidosubthalamic pathway.^{2,19} In fact, we found a decrease in the SNpr firing rate after a unilateral STN lesion. Lesions^{8,10} or high frequency stimulation of the STN^{9,13,20,21} have been shown to improve Parkinsonian motor systems, presumably by reducing the activity of the basal ganglia output structures.^{17,22} The STN is an important component of the basal ganglia and is considered to play a key role in the control of the output structures of the system. According to the concept of motor circuit functional

organization, the motor nuclei of the thalamus [ventromedial nucleus (VM) and VL in the rat] are the last relay before the signals from the basal ganglia motor-regulating circuit enter the cortex.² Inhibition of the two output structures in the system would induce a decrease in the inhibitory action on the motor thalamus and consequently would induce an increase in the excitatory input to the cortex. According to the widely accepted functional organization of the basal ganglia, a change in the firing activity of the output structures of the system is believed to induce an increase in the tonic inhibitory influence exerted by these structures on the activity of motor thalamic nuclei, resulting in the deactivation of motor cortical areas.^{2,3} In 6-OHDA induced rats, a decrease in the firing rate of VL neurons was found. This result supports the notion that motor nuclei of the thalamus [VM and VL in the rat] work between the basal ganglia motor circuit and the cortex. Another study showed that STN HFS increased the firing rate of VL neurons.²³ Our results show that the intrastriatal D₁ receptor agonist SKF38393 decreased the mean firing rate in the SNpr of rats with 6-OHDA lesions. This result shows that D₁ receptor stimulation directly controls the pathway. However, this D₁ agonist had no significant effect in the other experimental groups. Concerning the possible role for D₁ receptors in the STN, it is interesting to note that we found that the D₁ agonist SKF38393

significantly increased the firing rate of STN neurons in 6-OHDA lesioned rats.²⁴ Augmentation of responses to D₁ receptor agonists in the STN of lesioned rats has been reported previously in studies measuring biochemical indexes of neuronal activation, such as, *c-fos* expression and glucose metabolism.²⁵ This results suggests that D₁ agonists can modify the expression of molecules and the electrophysiological activity of neurons of the indirect pathway.^{24,26} In fact, the systemic administration of D₁ agonist has more dramatic effects on STN neuronal activity than the administration of D₂ agonists.^{24,26} D₁ receptors are present on the striatal output neurons projecting to the substantia nigra pars reticulata and the entopeduncular nucleus.²⁷ It could be possible that the increased activity observed in STN neurons after dopamine application is mediated by the D₁ receptor subtype. Thus, it seems unlikely that the D₁ receptor plays an important role in regulating STN neuronal activity, but we cannot rule out this possibility. Consequently, it appears that the role of D₁ receptors in regulating STN neuronal activity has not been well substantiated. Several studies have demonstrated that the STN receives direct dopamine input from the SNc and that both D₁ and D₂ receptors are found in the STN.⁶ Thus, the activity of STN neurons can be influenced directly by dopamine and its agonist/antagonists. Therefore, we suppose that SKF38393 induces decreased SNpr output via STN and the

direct pathway in the 6-OHDA lesioned rat. The effects of SKF38393 on the firing rate of SNpr was not investigated in SNc + STN lesioned rats.

We investigated the effects of SKF38393 on the activity of the VL in the thalamus, which is supposed to receive direct GABAergic projections from the SNpr output. SKF38393 induced a decrease of hyperactivity in SNpr neurons resulting in an increase in the hypoactivity in VL neurons. In SNc + STN lesioned rats, SKF38393 induce an increase in firing rate in VL neurons, which contrasted with an unchanged firing rate in SNpr neurons. In fact, Kreiss et al. (1996) suggested that the excitatory effects of dopamine might be due to glutamate release caused by the stimulation of D₁ receptors located on nerve terminals of the corticosubthalamic pathway.^{28,29}

The administration of the D₂ receptor agonist Quinpirole increased the mean firing rate of neurons in the SNpr of intact rats. Zhu et al. (2002) reported that Quinpirole (10 μ M) increased the mean firing rate of STN neurons in normal slices.³⁰ These results suggest that dopamine exerts an excitatory influence on STN neuronal activity, most likely via the stimulation of D₂-like receptors.³⁰ In the 6-OHDA lesioned rat, Quinpirole reduced the GABAergic increase in SNpr neurons by D₂ receptor stimulation, which resulted in the increased output of VL neurons. By acting on striatal D₂ receptors, Quinpirole would reduce the GABAergic input to the globus

pallidus, thus inducing increased inhibitory input to the STN, which would result in the reduction of STN neuron activity. The ventrolateral nucleus (VL) is known to receive direct GABAergic projections from the output structures of the basal ganglia.^{2,3} The present study shows that STN lesions induced an increased firing rate in VL neurons. This result demonstrates that denervation of dopaminergic neurons induces a decreased firing rate in VL neurons, consequently STN lesioning reversed this reduction of firing rate in VL neurons. This result supports the effect of STN lesioning on the activity of the ventrolateral nucleus of the thalamus (VL), which is known to receive direct GABAergic projections from the output structures of the basal ganglia. Therefore, the effects of lesioning on STN neurons demonstrate that GABAergic output from the SNpr projects to the VL directly. This hypothesis is confirmed by a positron emission tomography study, using regional cerebral blood flow measurements in Parkinsonian patients. Limousin et al. (1997), showed that STN HFS, which produces a significant improvement in movement performance, was accompanied by an increase in cortical activity of, the supplementary motor area and the dorsolateral prefrontal cortex.¹³ Boraud et al. (2001)'s study found Gpi rate inhibitions in MPTP-treated monkeys caused by the D₁ agonist SKF38393.³¹ Moreover, there is some evidence that robust inhibition of Gpi in MPTP-treated primates is associated

with dopamine agonist-induced dyskinesias,³¹ suggesting that, in primates or rodents, doses of dopamine agonists, which produce such inhibitions of the basal ganglia output nuclei are not optimal for reversing the behavioral effects of midbrain dopamine depletion. In vitro studies have shown that D₁ class agonists generally inhibit evoked discharge of the neostriatal medium spiny neurons.³² Further evidence for the excitatory action of D₁ agonists comes from their ability to induce immediate early gene expression.²⁷ Thus, it could be possible that the increased activity observed in STN neurons after dopamine application is mediated by the D₁ receptor subtype.

2. Firing pattern

Recent reports have suggested that the neuronal firing pattern is modified in Parkinsonism rather than the mean firing rate of the output nuclei neurons.^{18,33} Our results shows that 35% of the SNpr neurons recorded from lesioned rats discharged action potential bursts, whereas burst neurons account for 24% in normal rats. The proportion of burst neurons in the SNpr of 6-OHDA lesioned rats was significantly reduced by STN lesions. These results suggest that the interruption of the indirect pathway by a STN lesion regularizes the SNpr neuron discharge pattern in normal, and in particular, in SNc-lesioned rats. Many studies have shown that STN neurons fire irregularly or in a bursting

pattern after dopamine depletion.^{34,35,36} Other studies have provided evidence that an irregular or burst firing pattern might correlate better than firing rate with signs of Parkinsonism.^{11,35} The administration of SKF38393 or Quinpirole slightly decreased the proportion of burst neurons in the SNpr in 6-OHDA lesioned rats. This results supports a hypothesis about bursting activity, namely, that change in the firing pattern of SNpr units is a consequence of striatal denervation and is mediated by the indirect pathway via the STN.^{18,34} In rats with 6-OHDA lesions of the nigrostriatal pathway, burst-firing patterns have been previously reported in the STN, SNpr, and GP neurons.^{18,35,38,39} From recent evidence, it appears that the STN participates in the genesis of the burst pattern activity of GP and SNpr neurons in rats with 6-OHDA lesions, and that STN lesions can reverse this abnormal spontaneous pattern.^{17,18,36} More recent data⁴⁰ from an *in vitro* model in which STN and GP were co-cultured without dopamine inputs (a model resembling a dopamine-deficient basal ganglia system) supported the finding that dopamine depletion increases burst activity in the STN. In the SNc + STN lesioned rats, SKF38393 or Quinpirole induced an increased proportion of burst neurons in SNpr neurons. This result suggests that the bursting pattern may originate in parts other than the STN. The persistence of relatively irregular SNpr activity after the administration of an STN lesion may be partially due to direct

pallidal and/or striatal inputs to the SNpr. Indeed, neurons firing in bursts have been reported in the globus pallidus of 6-OHDA-lesioned rats³⁵ and direct projections from the globus pallidus to the SNpr have also been described. On the other hand, burst activity may reflect an imbalance between the two striatal efferent pathways, which normally act in synergy to control locomotor activity.⁴¹ Alternatively, bursting activity could originate in other structures projecting to the subthalamic nucleus, such as, the sensorimotor cortex or the intralaminar thalamic nuclei.³³ In animal experiments, the activities of both the non-bursting unitary and burst cells were described in the VL.⁴² We found reduced proportions of burst neurons in 6-OHDA lesioned rats. These results showed that the increased burst neurons in the SNpr did not induce burst VL neurons. In normal rats, the percentage of burst neuron among the VL neuron is higher than among SNpr neurons. Some researchers believe that the change in firing pattern, rather than the tonic firing rate might be of primary importance in mediating the functional effects of dopamine on forebrain activity and motor behavior,⁴³ and the firing pattern has been related to limb tremor by some studies.^{10,16} However, the reduced proportion of burst neurons due to 6-OHDA lesioning does not support the notion that burst neurons are related to the Parkinsonian motor symptoms of PD. The mechanism of bursting genesis may be similar to that observed for dopaminergic cells *in vitro*, in

which glutamate generated bursts by means of NMDA receptors.⁴⁴ In any case, bursting patterns would seem to be associated with an increase in neurotransmitter release.³⁵ Moreover, this may further increase the action of SNpr neurons on thalamic target cells. In addition, more recent study³⁷ showed that the selective degeneration of dopamine fibers in the STN induced a significant change in the firing pattern of its neurons, and demonstrated that the loss of dopamine in the STN can, at least in part, be at the origin of the bursting activity of STN neurons. *In vivo* and *in vitro* physiological studies have shown that the firing patterns of the thalamic projection neurons are based on the two basic mode-transfers and on the oscillatory modes, depending on the intrinsic membrane properties.³⁹ Results from others have suggested that membrane depolarization of a few mV may convert an STN neuron from the burst to the regular single-spike pattern type.⁴⁵ The pathophysiological origin of bursting activity remains unclear. The modification of the membrane properties of the neurons, induced by the loss of dopamine, in the basal ganglia motor circuit, is supposed to result in the genesis of the bursting neurons. Therefore, the temporary stimulation of D₁ and D₂ receptors by dopamine agonists does not seem to reduce the bursting neurons.

. Conclusions

This study demonstrates that STN lesions and dopamine agonists decreased the hyperactivity of the firing rate of SNpr neurons, and resulted in an increased firing rate of VL neurons in 6-OHDA lesioned rats. Concerning the firing pattern, the STN lesion was found to have a dramatic effect on SNpr neurons, but SKF38393 and Quinpirole did not. This result suggests that STN lesions and dopamine agonists may have different roles in the pathophysiology of PD.

The pathophysiological significance of bursting activity remains obscure, and the ability of dopamine agonist to regularize output nuclei firing patterns may explain why dopamine agonists lose efficacy as PD progress.

References

1. DeLong, MR. Primates models of movement disorders of basal ganglia origin. Trends Neurosci 1990;13:281-5.
2. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders, Trends Neurosci 1989;12:366-75.
3. Alexander GE, Crutcher MD, DeLong MR. Basal ganglia thalamocortical circuits: parallel substrates or motor, oculomotor, 'prefrontal' and 'limbic' functions. Prog Brain Res 1990;85: 119-46.
4. Delfs JM, Vivian MC, Tom JP Subthalamic nucleus lesions: Widespread effects on changes in the expression induced by nigrostriatal dopamine depletion in rats. J Neurosci 1995;15(10):6562-75.
5. Hutchison WD, Levy R, Dostrovsky JO, Lozano AM, Lang AE. Effects of apomorphine in globus pallidus neurons in Parkinsonian patients. Ann Neurol 1997;42:767-75.
6. Anderson JJ, Chase TN, Engber TM. Differential effect of subthalamic nucleus ablation on dopamine D₁ and D₂ agonist-induced rotation in 6-hydroxydopamine-lesioned rats. Brain Res 1992;588:307-10.
7. Charlety PJ, Grenhoff J, Chergut K, DeLaChapelle B, Buda M.

Svenson TH, et al. Burst firing of mesencephalic dopamine neurons is inhibited by somatodendritic application of kynurebate. *Acta Physiol Scand* 1991;142:105-12.

8. 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.
9. 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;5:382-9.
10. Bergman H, Wichmann T, DeLong MR Reversal of experimental Parkinsonism by lesion of the subthalamic nucleus. *Science* 1990;249:1436-8.
11. 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.
12. Gao DM, Benazzouz A, Piallat B, Bressan K, Ilinsky IA, Kultas-Ilinsky K, et al. High-frequency stimulation of the subthalamic nucleus suppresses experimental resting tremor in the monkey.

Neuroscience 1999;88:201-12.

13. Limousin P, Krack P, Pollak P, Benazzouz A, Ardouin C, Hoffmann D, et al. Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. *New Eng J Med* 1998;339:1105-11.
14. Dormont JF. Patterns of spontaneous unit activity in the ventro-lateral thalamic nucleus of cats. *Brain Res* 1972;37:223-39.
15. Bunney BS, Chiodo LA, Grace A. Midbrain dopamine system. Electrophysiological functioning: a review and new hypothesis. *Synapse* 1991;9:79-94.
16. Hutchinson WD, Allan RJ, Opitz H, Levy R, Dostrovsky JO, Lang AE, et al. Neurophysiological identification of the subthalamic nucleus in surgery for Parkinson's disease. *Annals Neurol* 1998;44:622-8.
17. Burbaud P, Gross C, Benazzouz A, Coussemaque M, Bioulac B. Reduction of apomorphine-induced rotational behaviour by subthalamic lesion in 6-OHDA lesioned rats is associated with a normalization of firing rate and discharge pattern of pars reticulata neurons. *Exp Brain Res* 1995;105:48-58.
18. Murer MG, Riquelme LA, Tseng KY, Cristal A, Santos J, Pazo JH. D₁-D₂ dopamine receptor interaction: an in vivo single unit

electrophysiological study. *Neuroreport* 1997;8:783-7.

19. Miller WC, DeLong MR. Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of Parkinsonism. *The basal ganglia. II.* Carpenter MB, Jayaraman A, editors. Structure and function: current concepts, advances in behavioral biology, vol. 32. New York: Plenum; 1987. p.415-27.
20. Ehringer H, Hornykiewicz O. Verteilung von Noradrenalin und Dopamin(3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klin Wochenschr* 1960;38:1236-9.
21. Limousin P, Krack P, Pollak P, Benazzouz A, Ardouin C, Hoffmann D, et al. Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. *New Eng J Med* 1998;339:1105-11.
22. 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.
23. 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.

24. Krack P, Benazzouz A, Pollak P, Limousin P, Piallat B, Hoffmann D, et al. Treatment of tremor in Parkinson's disease by subthalamic nucleus stimulation. *Mov Disord* 1998;13(6): 907-14.
25. Ruskin DN, Bergstrom DA, Walters JR. Nigrostriatal lesion and dopamine agonists affect firing pattern of rodent entopeduncular nucleus neurons. *J Neurophysiol* 2002;88:487-96.
26. Kita H, Kitai ST. Efferent projections of the subthalamic nucleus in the rat: light and electron microscopic analysis with the PHA-L method. *J Comp Neurol* 1987;260:435-452.
27. Gerfen CR, Enghber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, et al. D₁ and D₂ dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 1990;250:1429-32.
28. Kreiss DS, Mastropietro CW, Rawji SS, Walters JR. The response of subthalamic nucleus neurons to dopamine receptor stimulation in a rodent model of Parkinson's disease. *J Neurosci* 1997;17:6807-19.
29. Kreiss DS, Anderson LA, Walters JR. Apomorphine and dopamine D₁ receptor agonists increase the firing rates of subthalamic nucleus neurons. *Neuroscience* 1996;72:863-876.

30. Zhu Z, Bartol M, Shen K, Johnson SW. Excitatory effects of dopamine on subthalamic nucleus neurons: in vitro study of rats pretreated with 6-hydroxydopamine and levodopa. *Brain Res* 2002;945:31-40.
31. Boraud T, Bezard E, Bioulac B, Gross C. Dopamine agonist-induced dyskinesias are correlated to both firing pattern and frequency alterations of pallidal neurons in the MPTP-treated monkey. *Brain* 2001;124:546-57.
32. Levy R, Hazrati LN, Herrero MT, Vila M, Hassani OK, Mouroux M, et al. Re-evaluation of the functional anatomy of the basal ganglia in normal and Parkinsonian states. *Neuroscience* 1997;76:225-43.
33. Rohlf A, Nikkhah G, Rosenthal C, Rundfeldt C, Brandis A, Samii M, et al. Hemispheric asymmetries in spontaneous firing characteristics of substantia nigra pars reticulata neurons following unilateral 6-hydroxydopamine lesion of the rat nigrostriatal pathway. *Brain Res* 1997;761:352-6.
34. Hassani OK, Mourouz M, Feger J. Increased subthalamic neuronal activity after nigral dopaminergic lesion independent of disinhibition via the globus pallidus. *Neuroscience* 1996;72:105-15.
35. Hollerman JR, Grace AA. Subthalamic nucleus cell firing in the 6-

OHDA-treated rat: basal activity and response to haloperidol. *Brain Res* 1992;590:291-9.

36. Ni ZG, Bouali-Benazzouz R, Gao DM, Benabid AL, Benazzouz A. Time-course of changes in firing rates and firing pattern of subthalamic nucleus neuronal activity after 6-OHDA-induced dopamine depletion in rats. *Brain Res* 2001;899:142-7.
37. Tseng KY, Kasanetz F, Kargieman L, Pazo JH, Murer MG, Riquelme LA. Subthalamic nucleus lesions reduce low frequency oscillatory firing of substantia nigra pars reticulata neurons in a rat model of Parkinson's disease. *Brain Res* 2001;904:93-103.
38. Burbaud P, Gross C, benazzouz A, Coussemacq M, Bioulac B. Reduction of apomorphine-induced rotational behaviour by subthalamic lesion in 6-OHDA lesioned rats is associated with a normalization of firing rate and discharge pattern of pars reticulata neurons. *Exp Brain Res* 1995;105: 48-58.
39. Ni ZG, Bouali-Benazzouz R, Gao DM, Benabid AL, Benazzouz A. Intrastubthalamic injection of 6-hydroxydopamine induces changes in the firing rate and pattern of subthalamic nucleus neurons in the rat, *Synapse* 2001;40:145-53.
40. Plenz D, Kitai ST. A basal ganglia pacemaker formed by the

subthalamic nucleus and external globus pallidus. *Nature* 1999;400:677-82.

41. Paul ML, Graybiel AM, David JC, Robertson HA. D₁-like and D₂-like Dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson disease. *J Neurosci* 1992;12:3729-42.
42. Pan HS, Walters JR. Unilateral lesion of the nigrostriatal pathway decrease the firing rate and alters the firing pattern of globus pallidus neurons in the rat. *Synapse* 1988;2: 650-6.
43. Walters JR, Bergstrom DA, Molnar L, Freeman LE, Ruskin DN. Effects of dopamine receptor stimulation on basal ganglia activity. In: Kultas-Ilinsky K and Ilinsky IA, editors. *Basal Ganglia and Thalamus in Health and Movement Disorders*. New York: Kluwer Academic/Plenum, 2001. p.135-50.
44. Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: bursting firing. *J Neurosci* 1984;4:2866-76.
45. Beurrier C, Congar P, Bioulac B, Hammond C. Subthalamic nucleus neurons switch from single-spike activity to burst-firing mode, *J Neurosci* 1999;19:599-609.

6 - Hydroxydopamine

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D₁

D₂

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D₁

SKF38393

D₂

Quinpirole

.

(SNpr) (VL)

가 Kainic acid

(firing rate)

(firing pattern) ,

(regular non bursting pattern) (bursting pattern)

SKF38393

(26 ± 2.3 spikes/s 19 ± 2.9 spikes/s),

(4 ± 2.2 spikes/s 7 ± 0.8 spikes/s)

가 . 가 .

Quinpirole SNpr (29 ± 1.9

spikes/s 16 ± 0.4 spikes/s), VL 가 (5 ± 0.7

spikes/s 13 ± 3.3 spikes/s).

가 . , 가

. SKF38393 Quinpirole 가

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, SKF38393 Quinpirole

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: 6 - OHDA, , , , kainic acid,