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# Dopamine agonist-induced dyskinesias are correlated to both firing pattern and frequency alterations of pallidal neurones in the MPTP-treated monkey

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## Summary

**Despite the importance and frequency of levodopa-induced dyskinesias, little is known about their causal mechanisms. In this study, electrophysiological single-unit recordings of the neuronal activity of the globus pallidus internalis (GPi), the main basal ganglia output structure, and the globus pallidus externalis (GPe) were recorded continuously in both normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treated subhuman primates before and after the administration of three dopamine agonists—apomorphine (a dopaminergic mixed agonist), SKF-38393 (a $D_1$ partial agonist) and piribedil (a $D_2/D_3$ agonist)—at doses known to induce dyskinesias in the parkinsonian monkey. Changes in both the firing frequency and the firing pattern were analysed in relation to behavioural modifications. In both the normal and the parkinsonian monkey, the three agonists induced a decrease in the mean firing frequency of GPi neurones, although dyskinesias were induced only in the parkinsonian animals. In this situation, the improvement of parkinsonian motor abnormalities was correlated with the decrease in GPi firing frequency, whereas firing pattern changes were concomitant with the onset of dyskinesias. Moreover, firing frequency seemed to be decreased excessively during dyskinesias. The results indicate that the electrophysiological mechanism of dyskinesia involves an excessive decrease in GPi firing frequency and a modification of the firing pattern. However, the similarity between the induced decrease in firing frequency in normal and parkinsonian animals underlines the need for dopamine depletion in the induction of dyskinesias.**

**Keywords**: single-unit recordings; Parkinson's disease; globus pallidus; firing frequency

**Abbreviations**: GPe = globus pallidus pars externalis; GPi = globus pallidus pars internalis; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

## Introduction

The discovery that the death of nigrostriatal dopaminergic neurones is responsible for the debilitating motor syndrome observed in Parkinson's disease has led to the development of replacement therapies. Levodopa remains the standard treatment for Parkinson's disease, although it induces long-term side-effects. The most common of these, dyskinesia, affects nearly 50% of patients after a duration of illness of 5 years (Marsden *et al*., 1982), but its causal mechanisms remain unknown. Dopamine acts on striatal neurones through the $D_1$ and $D_2$ families of dopamine receptors. The $D_1$ family is subdivided into $D_1$ and $D_5$ receptors, and the $D_2$ family into $D_2$, $D_3$ and $D_4$ receptors (Sokoloff and Schwartz, 1995). Receptors of the $D_1$ family are thought to be located on neurones projecting to the globus pallidus pars internalis (GPi), whereas those of the $D_2$ family are found on neurones projecting to the pars externalis of the globus pallidus (GPe) (e.g. Gerfen *et al*., 1990; Le Moine and Bloch, 1995). Although the role played by these different receptors in the emergence of dyskinesia remains controversial (Bedard *et al*., 1999), the main hypothesis is that dyskinesia results from overstimulation of $D_1$ receptors, and the administration of a selective $D_2$ agonist should correct this imbalance (Durif,

1999). However, administration of a $D_2$ agonist has been reported as being able to induce dyskinesia in levodopa-naive monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Gomez-Mancilla and Bedard, 1992).

Whereas the electrophysiological investigation of dyskinesia is obviously difficult at the striatal level, some investigators are taking an interest in recording GPi activity, as this is the main basal ganglia output structure receiving striatal information through the direct and indirect pathways (Alexander and Crutcher, 1990; DeLong, 1990). The administration of levodopa and apomorphine to MPTP-treated monkeys (Filion *et al*., 1991; Boraud *et al*., 1998; Papa *et al*., 1999) or to parkinsonian patients (Hutchinson *et al*., 1997; Stefani *et al*., 1997; Merello *et al*., 1999*a*, *b*; Lozano *et al*., 2000) has been shown to reduce GPi firing frequency to a level considerably lower than in the normal animals, even in the absence of dyskinesia. Conversely, deep-brain stimulation and the administration of riluzole, both of which are known to improve the parkinsonian motor syndrome without inducing dyskinesia (Benazzouz *et al*., 1995; Gross *et al*., 1997), restore GPi activity close to the normal level (Boraud *et al*., 1996, 2000*b*).

A correlation between an excessive decrease in GPi discharge frequency and the appearance of levodopa-induced dyskinesia has been established recently in the MPTP-treated monkey by Papa and colleagues (Papa *et al*., 1999). However, these investigators focused only on the change in firing frequency, although modifications in the neuronal firing pattern have also been hypothesized to be involved (Obeso *et al*., 2000). Moreover, although this correlation affords significant new insights, Papa and colleagues restricted their study to the effect of levodopa. Thus, it has not yet been possible to clarify what role the direct pathway may have in the genesis of levodopa-induced dyskinesias. To do this, it would be necessary to look at the effects of $D_1$ and $D_2$ receptor agonists on the discharge characteristics of both parts of the pallidal complex (GPe and the GPi).

Therefore, we studied the effect of the administration of prodyskinetic doses of three dopaminergic agonists—apomorphine (a mixed $D_1/D_2$ agonist), SKF-38393 (a $D_1$ agonist) and piribedil (a $D_2/D_3$ agonist)—on the single-unit activity of pallidal neurones in normal and MPTP-treated monkeys.

## Material and methods

### *Animals*

Experiments were conducted on two female cynomolgus monkeys (*Macaca fascicularis*; CRP, Port Louis, Mauritius) aged 3 and 3.5 years and weighing 2.7 and 3.1 kg, respectively. A third cynomolgus monkey, matched for age, sex and weight, was used for histological controls. The animals were housed in individual primate cages under controlled conditions of humidity (50 ± 5%), temperature (24 ± 1°C) and light (12 h light/12 h dark cycle), food and water were available *ad libitum*, and the care of the animals was supervised by veterinarians skilled in the health-care and maintenance of non-human primates. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals. All efforts were made to minimize animal suffering and to use the smallest number of animals necessary to produce reliable scientific data. The animals were killed at the end of the experiments, and histochemical investigation of the brains showed a marked decrease in the number of tyrosine hydroxylase-immunoreactive neurones (method of Bezard *et al*., 1997) in the substantia nigra of both experimental monkeys (78.2 and 81.1% reduction) compared with the control values in the third, untreated monkey.

### *Surgery and MPTP administration*

A recording chamber was attached stereotactically under general anaesthesia [ketamine hydrochloride 10–15 mg/kg i.m. (intramuscularly) (Panpharma, Fougères, France) and xylazine 1.5–2.5 mg/kg i.m., Sigma, St Louis, MO, USA] at an angle of 45° to the sagittal plane to facilitate the positioning of the microelectrodes, which were then inserted parallel to the central axis of the chamber, as described previously (Boraud *et al*., 1996, 1998, 2000*a*). Monkeys were made severely parkinsonian by bilateral intracarotid injection of an acute dose of MPTP (0.8 mg/kg each side) under arteriographic control, as described previously (Benazzouz *et al*., 1993).

### *Behavioural assessment*

The animals' behaviour was assessed on a clinical scale for parkinsonian monkeys (Imbert *et al*., 2000), which rates the following symptoms of parkinsonian disability: tremor (0–3); variation in the general level of activity (0–3); body posture (flexion of spine, 0–3); vocalization (0–2); freezing (0–2); and frequency of arm movements (reaching for food, 0–3 for each upper limb). Rigidity (0–3 for each upper limb) was assessed at the end of each session in order not to interfere with assessment of the general level of activity. The maximum disability score was 25. Dyskinesias were also rated on the scale published by Benazzouz and colleagues (Benazzouz *et al*., 1995). The following items were assessed: frequency (0–3); nausea (0–1); and overall level of activity (from –2 to +2). The normal score was 0, the minimum dyskinesia score –2 and the maximum dyskinesia score 6. The clinical modifications induced by each drug were assessed (i) during regular sessions of direct behavioural evaluation and (ii) during each electrophysiological recording session, immediately after each recording. In the assessments made during the regular sessions of direct behavioural evaluation, two examiners observed the animal moving freely around its cage and evaluated its motor performance, coaxing it to

perform various tasks by offering appetizing fruit, and a third examiner, watching a simultaneous video recording, made a blind, independent assessment. Rating notes were compared regularly in order to eliminate observer bias (Taylor *et al*., 1994). In the assessments made during each electrophysiological recording session, the clinical evaluation was limited to the rigidity and arm movement items of the disability scale, plus the dyskinesia scale. The evaluation was made every 5 min and continued until performance scores returned to preinjection levels.

### *Electrophysiological recording*

Extracellular unit recordings were performed in calm, awake monkeys, in the absence of voluntary movements, as described previously (Boraud *et al*., 1996, 1998, 2000*a*, *b*). Animals were seated in a recording chair and tungsten electrodes (FHC, 6–8 MΩ) were lowered using a microdrive (OM 951, Narishige, Tokyo, Japan). Each recording lasted ~10 s (2000 interspike intervals). After signal amplification through a differential preamplifier (Model 113; Princeton Applied Research, Princeton, Mass., USA) and spike discrimination (N-750 Spike Analyser; Mentor), data were processed on-line through a computer (Power PC 6400; Apple Cupeatine, Calif., USA) programmed to stock sequences of interspike intervals via a custom-made interface. A time interval histogram was constructed from each recording. We then charted a density histogram for each cell, according to the method described by Kaneoke and Vitek (Kaneoke and Vitek, 1996), to determine the firing pattern (random, regular or bursting) as described previously (Boraud *et al*., 1998). Marking lesions were made by passing DC (30 μA for 10 s) through the recording electrode at selected points. These were then used, together with electrophysiological landmarks and the dark lines of gliosis that indicated recording tracks in the pallidal complex, to retrace the anatomical path of electrode penetration and the locations of the neurones that were recorded (data not shown).

The activity of the neurone was first isolated from background noise, and its activity was recorded three times over a period of ~20 min before the injection of any drug; this gave us a basal value for each drug. After injection of the agonist, the same neurone was then recorded every 5 min until it returned to the basal value. The value of *n* for each pharmacological situation indicates the number of fully evaluated neurones, i.e. approximately 40–50% of the total number of recorded cells in the normal situation and only 10–15% in the MPTP situation. The three recordings that deviated most from the basal value were pooled to give the 'best effect' value for each drug. The 'on' value, where applicable, was the mean firing frequency recorded during the period when the animal presented clinical amelioration without dyskinesia. The 'dyskinesia' value was the mean firing frequency recorded during the period when the animal presented dyskinesia. For the 'on' and 'dyskinesia' values, results were pooled for all three agonists.

### *Drugs*

Apomorphine (Apokinon®; Aguettant, Lyon, France), a non-selective agonist of dopamine receptors, was injected subcutaneously at a dose (0.1 mg/kg) known to induce dyskinesia in MPTP-treated animals (Kuno, 1997). SKF-38393 (RBI, Natick, Mass., USA), an agonist of the $D_1$ receptor, was administered intramuscularly at a dose (1.5 mg/kg) known to induce hyperkinesia in MPTP-treated animals (Jenner, 1995). Piribedil (Trivastal®, Eutherapie, Neuilly sur Seine, France), an agonist of $D_2/D_3$ receptors, was injected intramuscularly at a dose (3 mg/kg) known to induce dyskinesia in MPTP-treated animals (Smith *et al*., 1996). After the first set of tests with these drugs, the animals were treated with MPTP as described above to render them severely parkinsonian. The tests were then repeated with the same drugs.

### *Data analysis*

A one-way ANOVA (analysis of variance) with repeated measures was used to compare mean firing frequencies. When significant, the ANOVA was followed by a *post hoc* Dunnett multiple comparison test. The results for the two animals were pooled after the ANOVA had shown they were not different ($P > 0.5$). The 'best effect' values of each agonist on firing frequency in 'normal' animals and after MPTP treatment were compared using Student's *t* test. Mean 'on' values (firing frequency during the period when the animal presented a clinical amelioration without dyskinesia) and mean 'dyskinesia' values (firing frequency during dyskinesia) were pooled for all three agonists and compared using the paired *t* test. In order to compare firing patterns, we analysed the frequency of distribution of the different patterns ($\chi^2$ (2), $P < 0.05$ when $\chi^2 > 5.991$) according to the method described by Mushiake and colleagues (Mushiake *et al*., 1991). This comparison was made for each 2000-spike recording.

A correlation matrix was then built for each agonist and each structure (GPi and GPe) to identify possible correlations between clinical and electrophysiological parameters. For each recording, we calculated the correlation coefficient for the following variables: (i) the number of neurones whose mean firing frequency was significantly modified compared with the normal state; (ii) the number of neurones with a significantly modified firing pattern; (iii) the number of times the animal presented a significant clinical modification; and (iv) the number of times dyskinesias were observed. Items were considered correlated when $P < 0.05$.

## Results

### *Before MPTP administration*

As expected (Boyce *et al*., 1990; Nutt, 1990; Durif, 1999), no noticeable behavioural change or dyskinesia was observed for any of the agonists tested, whereas there were

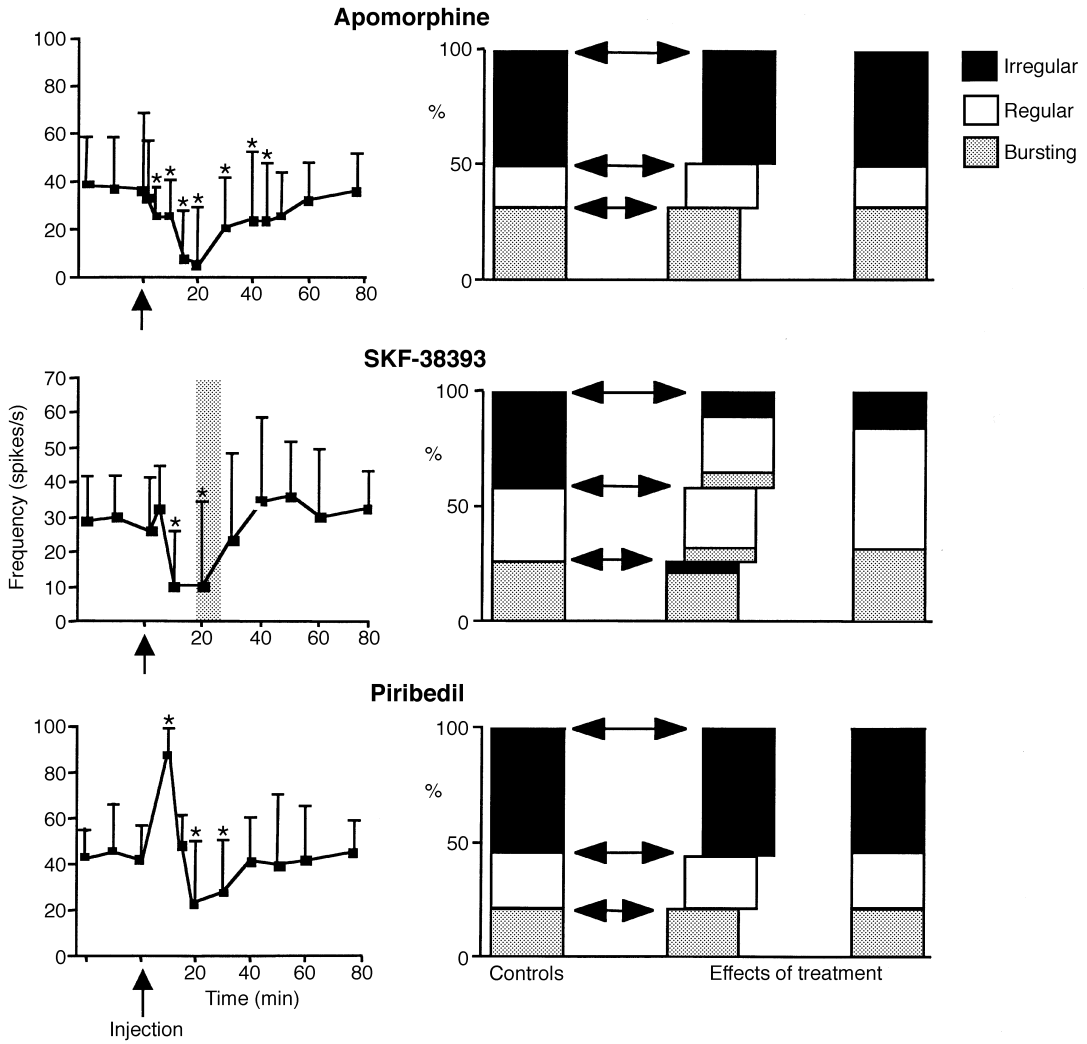


**Fig. 1** Response of GPe neurones to dopamine agonists in normal animals: *top*, apomorphine (mixed $D_1/D_2$ agonist) ($n = 16$); *middle*, SKF-38393 ($D_1$ agonist) ($n = 19$); *bottom*, piribedil ($D_2/D_3$ agonist) ($n = 24$). The panel on the left shows the effects on mean frequency of discharge. Vertical bars represent the standard deviation. An asterisk indicates a significant change from control frequency ($P < 0.05$). The shaded zone represents the duration of the period during which firing activity was modified. *Right*: this panel is a graphic representation of the percentage distribution of the different firing patterns. The left-hand columns show results before injection and the middle and right-hand columns show results obtained after injection. The middle columns represents the modifications induced in each neurone subpopulation of the GPe by the different agonists. The first step (bottom section) shows the modification of the firing pattern of cells discharging in bursts before injection; the second step (middle section) shows the modification of the firing pattern of cells discharging regularly before injection; and the third step (top section) shows the modification of the firing pattern of cells discharging randomly before injection. The right-hand columns show pooled data for the three subpopulations represented in the middle columns.

modifications in the electrophysiological activity of the pallidal complex.

## *GPe (Fig. 1)*

Mean firing frequency [$F(13,223) = 38.5$, $P < 0.0001$, $n = 16$ cells] decreased significantly 5 min after administration of the non-selective dopaminergic agonist apomorphine ($P < 0.05$) and recovered its normal level 45 min later. There was no modification of the firing pattern.

Injection of the $D_1$ agonist significantly decreased mean firing frequency [$F(13,265) = 48.7$, $P < 0.0001$, $n = 19$ cells] 10 min after administration ($P < 0.05$). The firing pattern was also significantly modified (increase in the number of regular neurones) for 10 min ($P < 0.05$). The effects lasted a total of 30 min.

The $D_2/D_3$ agonist induced a significant increase in mean firing frequency [$F(13,335) = 41.2$, $P < 0.0001$, $n = 24$ cells] 10 min after administration. This increase ($P < 0.05$) lasted 10 min and was followed by a sharp decrease lasting 20 min. The effects lasted a total of 40 min. There was no modification of the firing pattern.

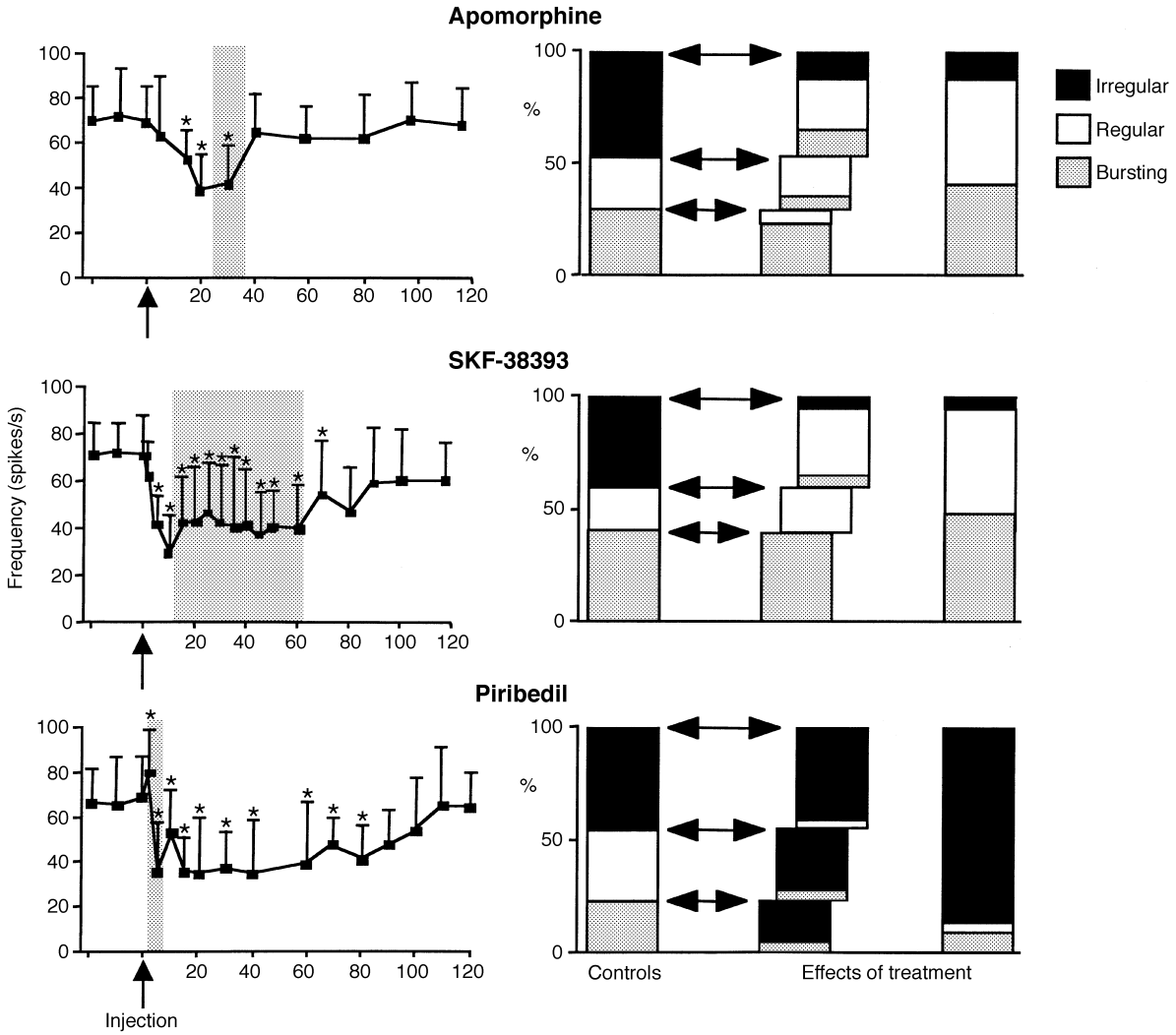


**Fig. 2** Response of GPi neurones to dopamine agonists in normal animals: *top*, apomorphine (mixed $D_1/D_2$ agonist) ($n = 16$), *middle*, SKF-38393 ($D_1$ agonist) ($n = 19$); *bottom*, piribedil ($D_2/D_3$ agonist) ($n = 22$). Details are as for Fig. 1.

## *GPi (Fig. 2)*

Mean frequency [$F(15,225) = 37.1$, $P < 0.0001$, $n = 16$ cells] decreased significantly 15 min after injection of the non-selective dopaminergic agonist ($P < 0.05$). The firing pattern presented a significant modification (increase in the number of regular neurones) 10 min later ($P < 0.05$). These effects lasted a total of 50 min.

Administration of the $D_1$ agonist also affected mean frequency [$F(17,341) = 27.1$, $P < 0.001$, $n = 19$ cells], which decreased significantly 5 min after injection ($P < 0.05$). This was followed 5 min later by a significant modification (increase in the number of regular neurones) of the firing pattern ($P < 0.05$), which lasted 45 min. The effects lasted a total of 70 min.

The $D_2/D_3$ agonist induced a significant transient increase in mean frequency [$F(25,571) = 102.1$, $P < 0.0001$, $n = 22$ cells] 5 min after administration ($P < 0.05$). This was followed 5 min later by a significant change (increase in the number of random neurones) in the firing pattern ($P < 0.05$). The increase in firing frequency was quickly followed by a significant decrease ($P < 0.01$) and the firing pattern returned to normal. Firing frequency was modified for a total of 80 min.

## *After MPTP administration*

Parkinsonian motor abnormalities were rated 15.2 ± 3.8 in MPTP-treated animals and were diminished by all three agonists (apomorphine, 5.2 ± 1.3; SKF-38393, 8.0 ± 2.5; piribedil, 5.6 ± 1.7). The dopaminergic agonists induced dyskinesias as expected with the doses used (apomorphine, 3.2 ± 0.7; SKF-38393, 0.9 ± 0.7; piribedil, 2.3 ± 1.0).

## *GPe (Fig. 3)*

Mean firing frequency [$F(17,143) = 23.1$, $P < 0.0001$, $n = 8$ cells] increased significantly 5 min after injection of apomorphine. This was followed 5 min later by a significant change (increase in the number of regular neurones) in the firing pattern ($P < 0.05$). The effects stopped after 20 min. 'Best effect' values were significantly

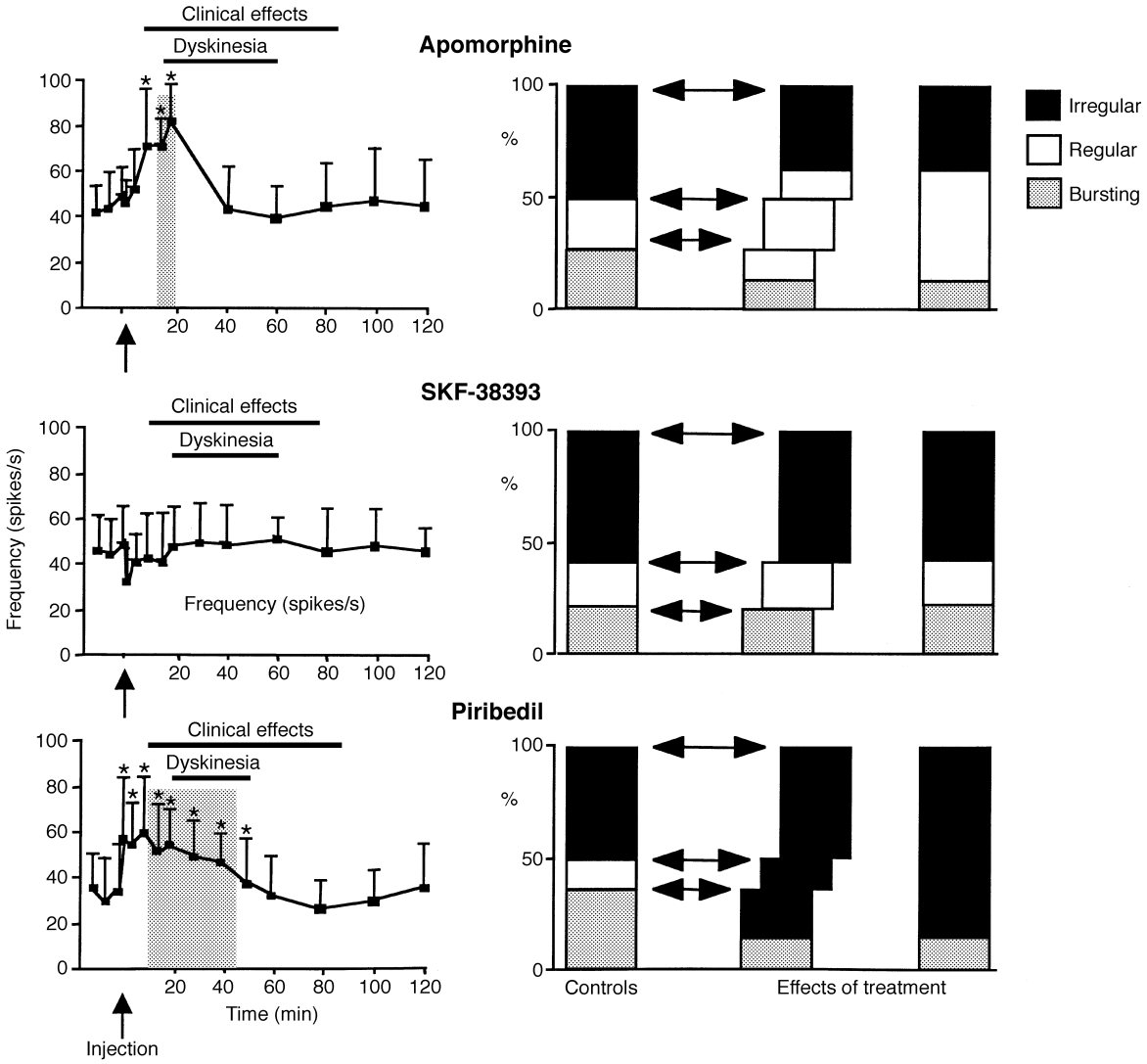


**Fig. 3** Response of GPe neurones to dopamine agonists in MPTP-treated animals: *top*, apomorphine (mixed $D_1/D_2$ agonist) ($n = 8$); *middle*, SKF-38393 ($D_1$ agonist) ($n = 5$); *bottom*, piribedil ($D_2/D_3$ agonist) ($n = 8$). *Left*: this panel shows the effect on mean frequency. Vertical bars represent the standard deviation. An asterisk indicates a significant change from control frequency ($P < 0.05$). The shaded zone represents the duration of the period during which the firing pattern was modified. The first horizontal line shows the duration of the clinical improvement; the second horizontal line shows the duration of dyskinesia. *Right*: this panel is a graphic representation of the percentage distribution of the different firing patterns. The left-hand columns show results before injection and the middle and right-hand columns show results obtained after injection. The middle column represents the modifications induced in each subpopulation of the GPi by the different agonists. The first step (bottom section) shows the modification of the firing pattern of cells discharging in bursts before injection; the second step (middle section) shows the modification of the firing pattern of cells discharging regularly before injection; and the third step (top section) shows the modification of the firing pattern of cells discharging randomly before injection. The right-hand columns show pooled data for the three subpopulations represented in the middle columns.

higher than those recorded before MPTP treatment ($P < 0.05$).

Administration of the $D_1$ agonist modified neither mean frequency nor firing pattern [$F(17,89) = 1.2$, $n = 5$ cells]. 'Best effect' values were significantly higher than those recorded before MPTP treatment ($P < 0.05$).

Injection of the $D_2/D_3$ agonist affected mean frequency [$F(19,159) = 17.8$, $P < 0.0001$, $n = 8$ cells], which increased significantly 10 min later ($P < 0.05$). This was followed 15 min later by a significant modification (increase in the number of random neurones) of the firing pattern ($P < 0.05$). The effects stopped after 50 min. 'Best effect' values were significantly higher than in animals before MPTP treatment ($P < 0.05$).

'On' values were not significantly different from 'dyskinesia' values in GPe neurones.

After injection of the non-selective agonist (Fig. 3, top) or the $D_2/D_3$ agonist (Fig. 3, bottom), no correlation was

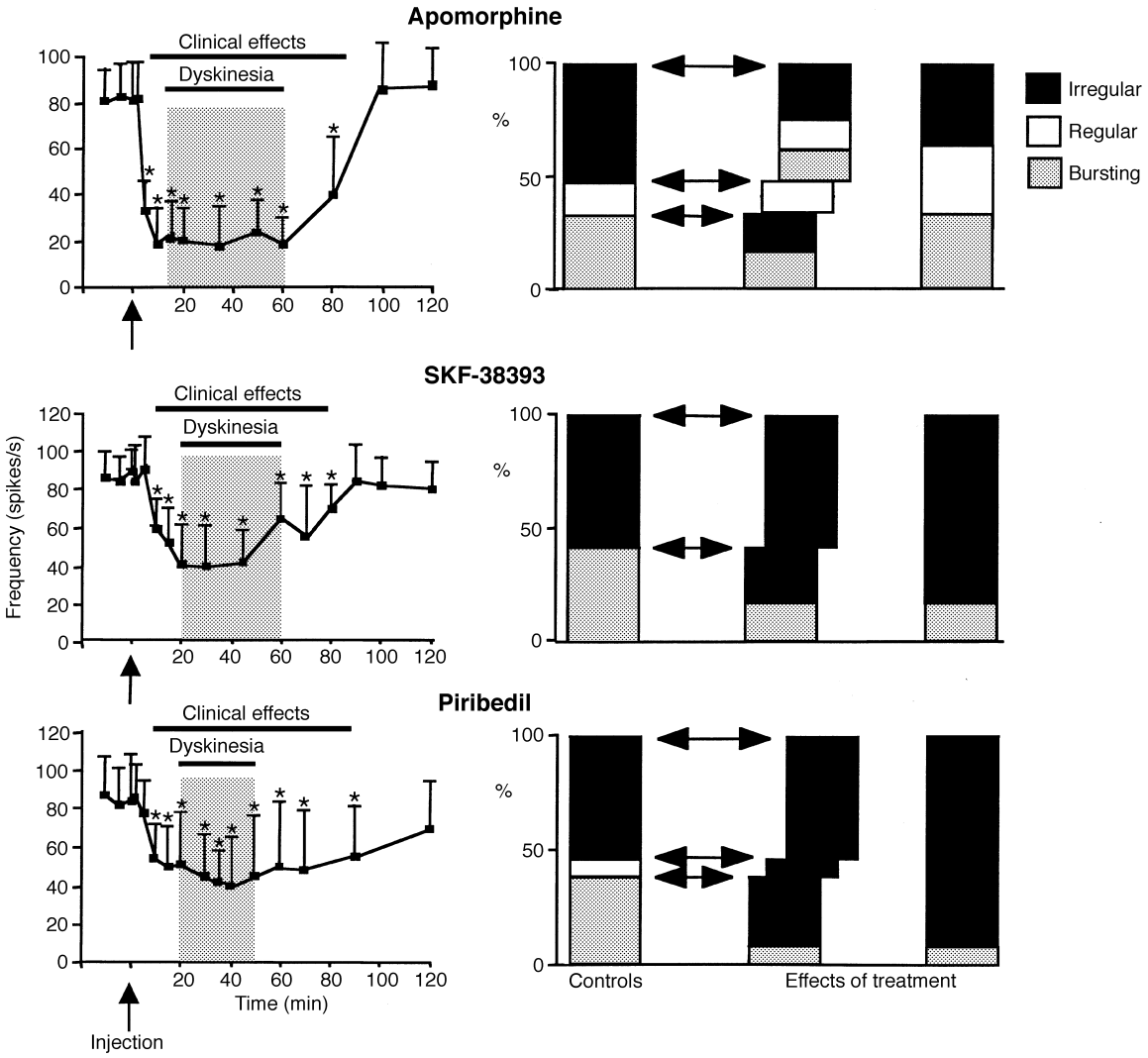


**Fig. 4** Response of GPi neurones to dopamine agonists in MPTP-treated animals: *top*, apomorphine (mixed $D_1/D_2$ agonist) ($n = 7$); *middle*, SKF-38393 ($D_1$ agonist) ($n = 11$); *bottom*, piribedil ($D_2/D_3$ agonist) ($n = 9$). Details are as for Fig. 3.

found between clinical improvement and the firing rate (respectively, $r = 0.3273$, $P < 0.05$ if $r > 0.5760$; $r = 0.41058$, $P < 0.05$ if $r > 0.4973$) or the firing pattern ($r = 0.2500$ and $r = 0.3303$, respectively) of GPe neurones. Nor was any correlation found between dyskinesia and the firing rate (respectively, $r = 0.2182$, $P < 0.05$ if $r > 0.5760$; $r = 0.3443$, $P < 0.05$ if $r > 0.4973$) or the firing pattern ($r = 0.0000$ and $r = 0.0006$, respectively).

## *GPi (Fig. 4)*

Administration of the non-selective dopaminergic agonist affected mean frequency [$F(23,167) = 57.4$, $P < 0.0001$, $n = 7$ cells], which decreased significantly 5 min later ($P < 0.05$). This was followed 10 min later by a significant modification (increase in the number of random neurones) of the firing pattern ($P < 0.05$). The effects stopped after 80 min. 'Best effect' values were significantly lower than before MPTP treatment ($P < 0.05$).

The $D_1$ agonist also induced a significant decrease in the mean firing frequency [$F(23,263) = 29.2$, $P < 0.0001$, $n = 11$ cells] 5 min after injection ($P < 0.05$). This was followed 15 min later by a significant modification (increase in the number of random neurones) of the firing pattern ($P < 0.05$). The effects stopped after 80 min. There was no significant difference between the 'best effect' values obtained before and after MPTP treatment.

The $D_2/D_3$ agonist significantly decreased mean frequency [$F(23,263) = 32.3$, $P < 0.0001$, $n = 9$ cells] 10 min after administration ($P < 0.05$). This was followed 10 min later by a significant modification (increase in the number of random neurones) of the firing pattern ($P < 0.05$). The

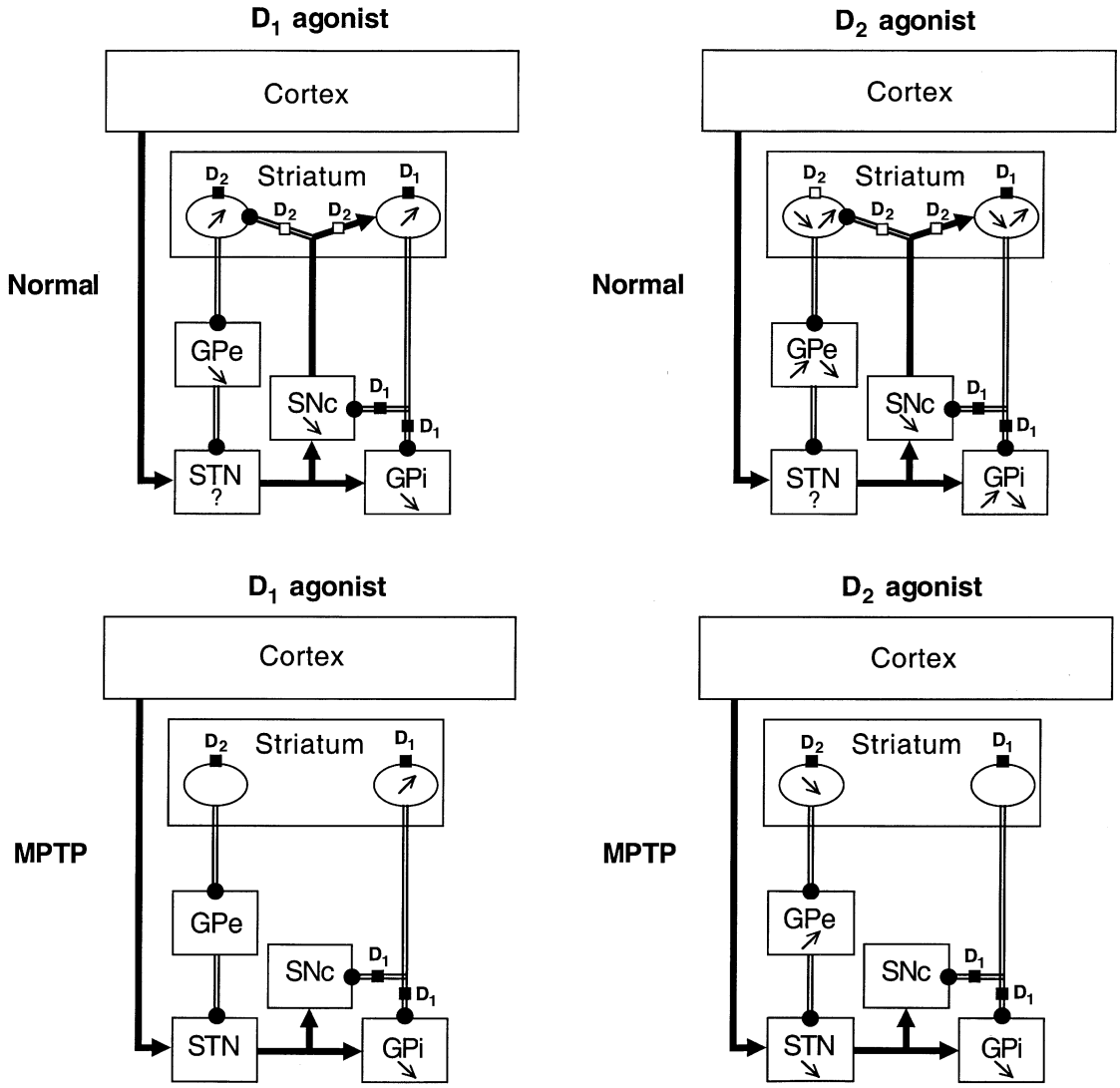


**Fig. 5** Schematic representation of interactions in the basal ganglia after treatment with $D_1$ and $D_2$ agonists, adapted from the scheme proposed by Le Moine and colleagues (Le Moine *et al.*, 1997). Variations in the electrophysiological activity of neurones compared with basal conditions are indicated by a small arrow within the structure or the neurone population. Dark lines represent excitatory pathways and white lines inhibitory pathways. STN = subthalamic nucleus; SNc = substantia nigra pars compacta.

effects stopped after 110 min. There was no significant difference between 'best effect' values obtained before and after MPTP treatment.

A significant difference was observed between 'on' and 'dyskinesia' values in GPi cells (49.5 ± 14.4 and 35.7 ± 17.6 Hz, respectively; $P < 0.05$).

Correlations were found between clinical improvement after injection of the non-selective agonist (Fig. 4, top), the $D_1$ agonist (Fig. 4, middle) and the $D_2/D_3$ agonist (Fig. 4, bottom) and modification of the firing frequency of GPi neurones (respectively, $r = 1$, $P < 0.05$ if $r > 0.5760$; $r = 1$, $P < 0.05$ if $r > 0.4973$; $r = 1$, $P < 0.05$ if $r > 0.4973$), but not modification of the firing pattern ($r = 0.5000$, $r = 0.4523$ and $r = 0.4714$, respectively). We found no correlation between dyskinesia after injection of the non-selective agonist (Fig. 4, top), the $D_1$ agonist (Fig. 4, middle) and the $D_2/D_3$ agonist (Fig. 4, bottom) and modification of the firing rate (respectively, $r = 0.5000$, $P < 0.05$ if $r > 0.5760$; $r = 0.5000$, $P < 0.05$ if $r > 0.5760$; $r = 0.4714$, $P < 0.05$ if $r > 0.4973$), whereas there was a correlation with modification of the firing pattern ($r = 1.00$, $r = 1.00$ and $r = 1.00$, respectively).

## Discussion

The present results shed light on the complex relationship between the clinical improvement and dyskinesia provoked by dopamine replacement therapy on the one hand and the electrophysiological parameters of globus pallidus neuronal activity on the other hand. This work confirms that a correlation existed between clinical improvement and decrease in the firing frequency of GPi neurones and that the

onset of dyskinesias was correlated with a modification of the firing pattern associated with an excessive decrease in firing frequency. Also, both receptor families may be involved in the genesis of dyskinesia, since both $D_1$ and $D_2$ agonists induced the above side- effects.

## *Methodological considerations*

Whether the changes in the electrophysiological activity of pallidal neurones we report are representative of the action of dopamine agonists on the whole pallidal complex is obviously critical to the significance of the present results. Baseline firing frequencies and pattern distributions of both the GPe and GPi neurones we recorded in normal and MPTP-treated monkeys are similar to those in recordings published previously (Filion and Tremblay, 1991; Bergman *et al*., 1994; Boraud *et al*., 1998, 2000*a*). The sample size of recorded neurones in each situation ensures the statistical validity of this comparison (GPe: normal, $n = 59$; MPTP, $n = 21$; GPi: normal, $n = 57$; MPTP, $n = 27$). However, whereas baseline characteristics represent a sample of the population of all pallidal neurones in the population of all monkeys, changes in neuronal activity induced by dopamine agonists can only be interpreted as representing a sample of cells from the populations within these two monkeys. Nevertheless, although the value of $n$ for each pharmacological agent may appear to be unsatisfactory, it indicates the number of fully evaluated neurones, i.e. from before dopamine agonist injection until return to baseline activity, which represents ~40–50% of the total number of recorded cells in monkeys before MPTP treatment and only 10–15% in monkeys after MPTP treatment.

## *Can the present data be explained by the current model of basal ganglia organization?*

The changes affecting the electrophysiological activity of the GPe in normal animals seem to contradict the current model of basal ganglia function, which postulates that there is no interaction between the direct $D_1$-dependent and the indirect $D_2$-dependent pathways. Indeed, both apomorphine (the non-selective agonist) and SKF-38393 (the $D_1$ agonist) induced a noticeable decrease in GPe firing frequency, whereas piribedil (the $D_2/D_3$ agonist) induced a short increase followed by a longer decrease. The data from MPTP-treated animals were, in contrast, consistent with the classical model of basal ganglia function. The $D_1$ agonist induced no modification of GPe discharge frequency, whereas both the non-selective agonist and the $D_2/D_3$ agonist induced an increase in the firing rate. These results are in accordance with data obtained from parkinsonian patients (Hutchinson *et al*., 1997; Stefani *et al*., 1997) and MPTP-treated animals (Filion *et al*., 1991). All three dopamine agonists induced, in the 'normal' animals, a decrease in the firing rate of GPi neurones. The slight biphasic increase in firing frequency observed after injection of the $D_2/D_3$ agonist was not reflected in any clinical effect. This may be explained by the fact that firing frequency did not reach a pathological level (Bezard *et al*., 1999). In MPTP-treated monkeys, all three agonists induced a marked decrease in GPi firing frequency. These results also confirm the data already obtained in humans and in MPTP-treated monkeys (Filion *et al*., 1991; Hutchinson *et al*., 1997; Stefani *et al*., 1997).

It thus appears that, whereas the segregation of a $D_1$ or direct pathway from a $D_2$ or indirect pathway would fit well with the pathological situation, it would not fit with the normal situation. Similar observations have led some authors to dispute the existence of $D_1/D_2$ segregation (Surmeier *et al*., 1996). However, it is plausible that systemic (subcutaneous or intramuscular) administration of dopamine agonists may act on different structures in the basal ganglia, given that dopamine receptors have been described in other basal ganglia nuclei (Mansour *et al*., 1990; Caillé *et al*., 1996; Levant, 1998; Hassani and Feger, 1999). The results obtained by Le Moine and colleagues may help to clarify the interpretation of our data. They showed that expression of c-*fos*, an index of the level of activity of a structure, can be activated through $D_1$ and inhibited through $D_2$ receptors in both striatal output pathways ('direct' and 'indirect') in normal rats (Le Moine *et al*., 1997). According to these authors, this paradoxical influence could be explained by the fact that the $D_1$ agonist acts at the level of the substantia nigra (Fig. 5) on presynaptic $D_1$ receptors located on GABAergic neurones connecting back to dopaminergic cells (Caillé *et al*., 1996). This would decrease dopaminergic release in the striatum, inducing disinhibition of the activity of $D_2$ neurones, which would, in turn, have an inhibitory influence at GPe level. This hypothesis fits in well with our results, as this effect was cancelled in our animals by dopaminergic depletion. The fact that, in the study of Le Moine and colleagues, the $D_2$ agonist induced a decrease in c-*fos* expression supports our observations. Indeed, they killed the rats 1 h after injection of the agonist (Le Moine *et al*., 1997), which corresponds to the first phase of our recordings. The later paradoxical decrease in firing frequency could be explained by the inhibitory influence that the $D_2$ agonist exercises on $D_2$ presynaptic receptors, decreasing dopaminergic inhibition of the GABAergic neurones of the indirect pathway, thus increasing the inhibitory input to the GPe.

## *Both $D_1$ and $D_2$ agonists induced dyskinesias*

Our results confirm that dopamine depletion is a necessary condition for the emergence of dyskinesia (Boyce *et al*., 1990; Nutt, 1990; Durif, 1999). Indeed, no behavioural changes were observed in the 'normal' monkeys, whereas in the MPTP-treated animals all three agonists induced dyskinesia. However, the dyskinesias provoked by SKF-38393 (the $D_1$ agonist) were less severe than those provoked by the non-selective agonist and the $D_2/D_3$ agonist. Because both $D_1$ and $D_2$ agonists appear to generate dyskinesia, it is

not possible, from our data, to posit a link between dyskinesia and a pharmacological stimulation of either the direct pathway or the indirect pathway. Such a correlation has been proposed (Blanchet *et al*., 1995, 1996) but other studies suggest that the mode of stimulation of dopamine receptors may be the most important factor. These reports indicate that continuous dopaminergic stimulation is best for the optimal regulation of the basal ganglia circuit (Goulet *et al*., 1996, 1997).

### *Dyskinesias are correlated to changes in GPi neuronal activity*

Whereas no correlation has been established between electrophysiological parameters of the GPe and clinical improvement, with or without dyskinesia, change in neuronal activity in the GPi has been linked to the genesis of dyskinesia. Indeed, there was a correlation with modification of the firing pattern, although this was not related to a specific pattern of discharge. Apomorphine-induced dyskinesia corresponded to an increase in the number of regular neurones, whereas with both SKF-38393- and piribedil-induced dyskinesia it was the number of random neurones that increased. Common to all three dyskinesias was the switch of a number of neurones from a bursting to a random pattern. This type of switch could play a key role in the genesis of dyskinesia (Obeso *et al*., 2000), although it is unlikely that this is the only mechanism involved.

Our results also support the correlation between dyskinesia and an excessive decrease in the firing frequency of GPi neurones. A similar excessive decrease was reported by Papa and colleagues (Papa *et al*., 1999) in MPTP-treated monkeys treated with levodopa and Lozano and colleagues (Lozano *et al*., 2000) in parkinsonian patients treated with apomorphine. Although Lozano and colleagues proposed an interesting model of dopaminergic drug-induced dyskinesias based on changes affecting mainly the firing frequency (Lozano *et al*., 2000), our present results stress the need to associate changes in firing frequency with changes in firing pattern in order to explain the occurrence of dyskinesia.

### *Limits of the 'excessive decrease' hypothesis*

This hypothesis should be treated with caution. If dyskinesias are linked to an excessive decrease in activity, this is in comparison with the 'best on' situation. All the previous studies have been done either in MPTP-treated monkeys (Filion *et al*., 1991; Boraud *et al*., 1998; Papa *et al*., 1999) or in parkinsonian patients (Hutchinson *et al*., 1997; Stefani *et al*., 1997; Merello *et al*., 1999*a*, *b*; Lozano *et al*., 2000). The present study is the first that has also recorded the effects of dopaminergic agonists on the pallidal activity of normal monkeys. These agonists did not induce any behavioural abnormality or dyskinesia in normal monkeys, but they did provoke a significant decrease in firing frequency. Although the firing frequencies of GPi neurones thus become similar in 'normal' and MPTP-treated animals whatever the agonist administered, the behaviours that are exhibited are far from similar. Therefore, the concept of excessive decrease in neuronal activity in the GPi should be used with caution, and only with reference to the decrease observed in the 'best on' situation. Thus, changes in pattern distribution would certainly play the crucial role in levodopa- and dopamine agonist-induced dyskinesias. This also questions the need for dopamine depletion in the emergence of dyskinesia. Further studies on the physiopathology of dyskinesias must take into account the present results, especially those regarding firing patterns, in order to elucidate the mechanisms of their origin.

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