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Distinct changes in evoked and resting globus pallidus activity in early and late Parkinson's disease experimental models

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Abstract

The main clinical manifestations of Parkinson's disease are caused by alterations of basal ganglia activity that are tied in with the progressive loss of mesencephalic dopaminergic neurons. Recent theoretical and modeling studies have suggested that changes in resting neuronal activity occurred later in the course of the disease than those evoked by phasic cortical input. However, there is no empirical support for this proposal. Here we report a marked increase in the responsiveness of globus pallidus neurons to electrical motor cortex stimulation, in the absence of noticeable changes in resting activity, in anesthetized rats that had consistently shown a deficit in forelimb use during behavioral testing before the experiments, and had ~45% dopamine neurons spared in the substantia nigra. Pallidal neurons were also over-responsive to motor cortex stimulation and lost spatial selectivity for cortical inputs in rats with extensive nigrostriatal damage. After partial lesions, over-responsiveness was mainly due to an increased proportion of neurons showing excitatory responses, while extensive lesions led to an increased likelihood of inhibitory responding neurons. Changes in resting neuronal activity, comprising pauses disrupting tonic discharge, occurred across different global brain states, including an activated condition which shares similarities with natural patterns of cortical activity seen in awake states and rapid eye-movement sleep, but only after massive nigrostriatal degeneration. These results suggest that a loss of functional segregation and an abnormal temporal encoding of phasic cortical inputs by globus pallidus neurons may contribute to inducing early motor impairment in Parkinson's disease.

Introduction

The effect of nigrostriatal lesions on basal ganglia resting neuronal activity has been the subject of numerous studies. Akinesia, the main clinical manifestation of Parkinson's disease, has been proposed to stem from sustained changes in average neuronal discharge rate in the subthalamic nucleus (STN) and basal ganglia output nuclei (Albin *et al*., 1989; DeLong, 1990), or from abnormal synchronous oscillations and other alterations in resting firing patterns (Bergman *et al*., 1998; Bevan *et al*., 2002; Hutchison *et al*., 2004). However, a recent computational modeling study suggests that changes in resting basal ganglia activity occur only after extensive nigrostriatal damage like that seen in advanced Parkinson's disease (Leblois *et al*., 2006a). Alternatively, it has been argued that, to gain insight into the mechanisms of akinesia, basal ganglia activity should be evaluated during voluntary movement rather than in resting conditions (Mink, 1996; Leblois *et al*., 2006a). However, as voluntary movement is severely impaired in Parkinson's disease, studies of this kind are scarce (Leblois *et al*., 2006b).

Models of basal ganglia dysfunction in Parkinson's disease, regardless of whether they emphasize alterations in resting activity or movement-related modulations of activity, presume that an increased gain of pathways conveying cortical activity through striatopallidal and subthalamic neurons contributes to produce akinesia (Crossman, 1987; Albin *et al*., 1989; DeLong, 1990; Mink, 1996; Hirsch *et al*., 2000; Bevan *et al*., 2002; Leblois *et al*., 2006a). Moreover, an increased functional convergence of cortical inputs on individual basal ganglia neurons (loss of spatial selectivity) may impede action selection (Mink, 1996; Bergman *et al*., 1998; Leblois *et al*., 2006a). Whether animals having partial dopamine neuron depletion and moderate parkinsonian-like motor deficits, reminiscent of early-stage Parkinson's disease, experience any of these changes in basal ganglia activity remains uncertain.

The rat globus pallidus (GP; rodent homolog of primate external pallidal segment) is connected with all basal ganglia structures, mainly reciprocally (Smith *et al*., 1998). Its most common neuronal type sustains autonomous pacemaker firing in the absence of functional synaptic input (Nakanishi *et al*., 1985; Kita & Kitai, 1991; Nambu & Llinas, 1994; Cooper & Stanford, 2000). *In vivo*, cortical drive, transmitted through striatopallidal and subthalamopallidal neurons, induces variability in GP activity (Ryan & Clark, 1991; Kita, 1992; Kita *et al*., 2004). Here, with the aim of simulating a dynamic activation of trans-striatal and trans-STN pathways to the GP, we examined the response of GP neurons to electrical stimulation of two motor cortex sites, in anesthetized rats that had consistently shown moderate or severe forelimb use impairment during behavioral testing before the experiments. We speculated that GP neurons would show

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an increased responsiveness to cortical stimulation in rats having conspicuous forelimb use impairment associated with partial nigrostriatal damage. Furthermore, we examined the spontaneous activity of GP neurons in the same rats, with the aim of determining if changes in resting activity become apparent only after extensive nigrostriatal damage.

Materials and methods

Animals were cared for in accordance with Argentine regulations (RS617/2002, Servicio Nacional de Sanidad y Calidad Agroalimentaria). Experimental protocols were approved by the Secretariat of Science of the School of Medicine of the University of Buenos Aires. Male adult Sprague–Dawley rats (190–220 g) were treated with desipramine (25 mg/kg, i.p.) to protect noradrenergic nerve terminals, anesthetized with chloral hydrate (400 mg/kg, i.p.), and injected with vehicle alone (0.1% ascorbic acid, 4 μL) or vehicle containing either 6 or 8 μg free base 6-hydroxydopamine (6-OHDA), in the left medial forebrain bundle, as described in Tseng *et al*. (2005). Rat performance in the stepping test was examined after 3 days, the third week after surgery, and once more the day before performing the electrophysiological recordings. We have previously reported that lesions performed with either 6 or 8 μg of 6-OHDA produced similar average deficits in the stepping test (Tseng *et al*., 2005). In the present study, in order to get two lesion groups exhibiting different degrees of motor impairment, we selected rats for electrophysiological recordings on the basis of performance in the stepping test, as follows: to be included in the 'severe deficit' or 'moderate deficit' lesion group, respectively, rats needed to exhibit, in every behavioral evaluation, fewer than two ($n = 9$ rats) or 3–7 ($n = 10$ rats) adjusting steps with the contralateral forelimb. Control rats ($n = 10$) performed 10–14 steps with either forelimb (Fig. 1A).

Four to six weeks after surgery, single GP units and the motor cortex local field potential (LFP) were simultaneously recorded under urethane anesthesia, following published protocols (Zold *et al*., 2007). Briefly, rats were anesthetized with urethane (1.2–1.5 g/kg, i.p.), treated with a local anesthetic in the scalp and pressure points (bupivacaine hydrochlorate solution, 5% w/v, 0.1–0.3 mL, infiltrated) and secured to a stereotaxic frame. Temperature was maintained between 36 and 37 °C with a servo-controlled heating pad. Additional urethane was given as required to retain an abolished reflex response to a tail pinch or pressure applied to the hind paw (customarily 0.1–0.2 g/kg i.p. every 2–4 h). Three concentric bipolar electrodes (SNE-100, Better Hospital Equipment, Rockville Centre, NY, USA) positioned in the motor cortex (Fig. 1B) allowed us to record differentially the LFP (0.1–300 Hz bandwidth) and to perform bipolar electrical stimulation at two motor cortex sites.

Extracellular recordings of GP neurons (0.8–1.2 mm caudal to bregma, 5–5.5 mm from midline with a 20° angle in the coronal

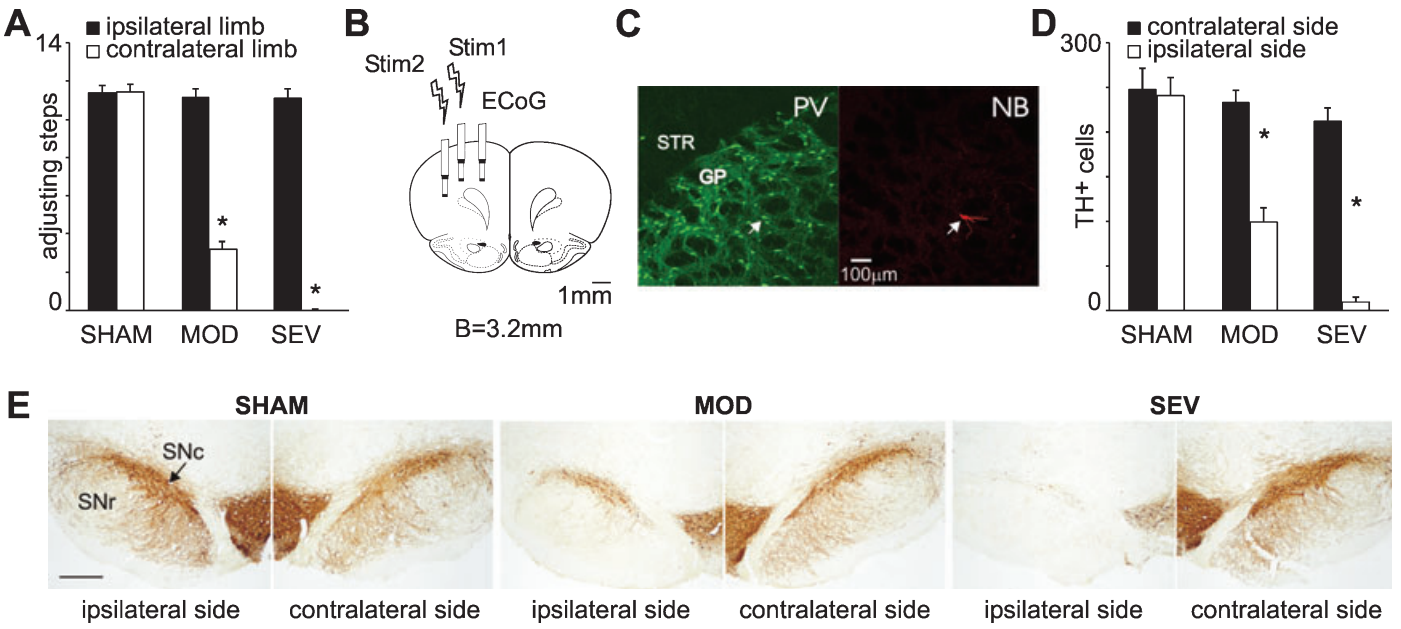

FIG. 1. Behavioural testing and post-mortem assessment of dopamine neuron loss. (A) Rats with 6-OHDA-induced nigrostriatal lesion were selected for the present study on the basis of their performance in the stepping test, so as to form two lesion groups having a moderate or severe deficit in contralateral forelimb use (*$P < 0.01$ vs. either sham rats or 'severe deficit' lesion group, Tukey's *post-hoc* test after a main interaction in a two-way ANOVA with repeated measures on side, $F_{2,26} = 151.4$, $P = 4.7 \times 10^{-15}$; data are mean ± SEM). (B) Four to 6 weeks after surgery, the rats were prepared for electrophysiological recording. Bipolar concentric electrodes were placed in the motor cortex so as to obtain a differential local field potential (ECoG) recording and to perform electrical stimulation (Stim 1, medial motor cortex; Stim 2, lateral motor cortex). (C) Single unit recordings of globus pallidus (GP) neurons were obtained with high-impedance glass electrodes, which allowed us to perform juxtacellular labeling with Neurobiotin (NB, red). As parvalbumin (PV, green) is present in most pallidosubthalamic neurons but only in a subset of striatal interneurons (Bolam *et al*., 2000), the border between the striatum (STR) and GP could be easily discerned in coronal sections immunostained with anti-PV antibodies (stereotaxic plane −0.92 mm; Paxinos & Watson, 1997). (D) Post-mortem counts of tyrosine hydroxylase (TH)-immunoreactive neurons in the substantia nigra pars compacta (SNc) confirmed different degrees of nigrostriatal degeneration in rats having moderate or severe deficit in the stepping test (*$P < 0.01$, vs. either sham rats or 'severe deficit' lesion group, Tukey's *post-hoc* test after a main interaction in a two-way ANOVA with repeated measures on side, $F_{2,21} = 34.3$, $P = 2.5 \times 10^{-7}$. data are mean ± SEM). Cell counts could be performed on seven sham, nine moderately akinetic and eight severely akinetic rats. (E) Coronal sections of the mesencephalon and forebrain stained with antibodies directed against TH, corresponding to a control rat (left) and representative rats displaying moderate (middle) or severe (right) impairment in the stepping test. Approximate coronal stereotaxic coordinates are −5.3 mm from Bregma, according to Paxinos & Watson (1997). Scale bar = 0.5 mm. Ipsilateral: to 6-OHDA injections. MOD: moderate deficit in contralateral forelimb use; SEV: severe deficit in contralateral forelimb use.

plane, 4–6 mm from the brain surface; Paxinos & Watson, 1997) plus juxtacellular cell labeling (Pinault, 1996) were performed with glass microelectrodes filled with 2% Neurobiotin (Vector Laboratories, USA) in 0.5 M NaCl (tip diameter, 1–2 μm; 14–25 MΩ), as described in Zold *et al*. (2007). The extracellular potential (0–10 kHz) was amplified (Axoclamp 2B, Axon Instruments, Foster City, CA, USA), digitized at 20 kHz (DigiData 1200, Axon Instruments) and digitally filtered (0.3–3 kHz, Axoscope 9.0, Axon Instruments). The microelectrode was moved forward until a single GP unit (signal-to-noise ratio typically > 3) could be isolated. Stable spontaneous activity was recorded for 3–5 min under the prevalent slow wave state of the cortical LFP (Kasanetz *et al*., 2006). Then, electrical stimulation was delivered to the medial motor cortex site. Four consecutive series of stimuli, consisting of 100 square wave pulses each (300-μs width, 2-s interpulse interval), at 300, 500, 700 and 900 μA, respectively, were delivered using a constant current isolator triggered by a pulse generator (Isolfex and Master-8, AMPI, Jerusalem, Israel). After recording an additional 3–5 min of stable spontaneous activity, the same protocol of stimulation was delivered to the lateral motor cortex site. The full protocol required ∼45 min of recording for each GP neuron. In some instances, at the end of the protocol, positive current pulses (250 ms long at 2 Hz), strong enough so as to drive spike discharges (3–15 nA), could be delivered through the recording glass electrode for 5–15 min, in order to load the neuron with Neurobiotin (Fig. 1C). Also, spontaneous activity could be recorded during episodes of cortical LFP activation, which can occur spontaneously in the urethane-anesthetized rat (see Kasanetz *et al*., 2006).

At the end of the experiments, rats were given a high dose of urethane and transcardially perfused with saline containing heparin (500 UI/L) followed by 4% paraformaldehyde in phosphate-buffer. To assess nigrostriatal damage, we counted tyrosine hydroxylase (TH)-immunoreactive (+) neurons in the substantia nigra pars compacta (SNc), as described in Tseng *et al*. (2005). Correct location of stimulation and recording sites in the frontal cortex was assessed in tissue sections stained with Safranine O. Pallidal sections from experiments during which GP cells were presumably loaded with Neurobiotin were processed for parvalbumin (PV) immunohistochemistry as described in Zold *et al*. (2007).

Synchronization between neuronal spike trains and cortical LFP, during resting states, was assessed by spectral analysis followed by coherence and phase estimations (Zold *et al*., 2007). Briefly, fast Fourier transforms were computed from 6.6-s (cortical slow wave) or 4-s (cortical activation) sliding time windows (passed through a Hanning window) with 25% overlap. Spectral densities were expressed as relative power within the range 0.25–32 Hz (resolution, 0.12 and 0.25 Hz, respectively; confidence limit settled at 95% of the inverse gaussian error function). A significant coherence (higher than $1 - (0.05)^{1/L-1}$, where L = number of segments) at the frequency of a significant peak in the cross-amplitude spectrum was taken as evidence of oscillatory synchronization (Halliday *et al*., 1995). Phase lags were calculated from phase spectra and corrected for the lag introduced by electronic filters as described in Tseng *et al*. (2001). To evaluate phase locking of spike discharges to cortical rhythmic activity during resting states, we computed the instantaneous phase of cortical LFP (i.e. phase angle of the Hilbert-transformed waveform; see Kasanetz *et al*., 2006) at the time of occurrence of every action potential in a spike train. The resulting collections of LFP instantaneous phases were depicted in circular plots, where the circular axis is the phase angle of cortical LFP and the radial axis the rate of occurrence of spike discharges. To estimate deviation from a circular uniform distribution ($P < 0.05$), which is an index of phase-locked activity (Siapas *et al*., 2005), we computed the Rayleigh probability (Fisher, 1993). By computing spike-triggered averages, we looked for synchronization between spike discharges and LFP events. Spike-triggered averages were computed on 0.6-s time windows centered at the time of the spike, and were considered significant when a peak or trough occurring within 0.1 s around the spike time deviated by more than one standard deviation from the average LFP amplitude (which was computed for the 0.2 s at the beginning and end of the 0.6-s time window). In addition, we studied the coefficients of variation and asymmetry ('skewness') of interspike interval histograms (ISIHs; built from trains with > 600 spikes), as an index of regularity in GP neuronal discharge during resting states. This was complemented by a study of 'burst' and 'pause' incidence by means of the surprise method of Legendy & Salcman (1985), which can be adapted to detect pauses (Elias *et al*., 2007). Briefly, the surprise of a burst or pause is a measure of how improbable it would be to find a given sequence of ISIs in a neuron discharging at a given average firing rate, assuming that firing follows a Poisson distribution. Pause detection started at an ISI three times longer than the average ISI, and burst detection at a sequence of three ISIs with half the duration of the average ISI. Then, the algorithm determines whether adding the ISI that precedes or follows the detected string increases its surprise, and loops until adding additional ISIs does not increase surprise further. Computations relating to the slow wave state were performed on 3–5-min signal segments, and those concerning spontaneous cortical activation on 30-s to 2-min episodes.

Responses to cortical stimulation were assessed in peri-stimulus time histograms (PSTHs), which were built from 100 stimulation trials using a 1-ms bin. A response was recorded if the number of spikes per bin increased over (excitation) or decreased below (inhibition) 75% of the average prestimulus (200 ms) spike per bin count, for at least three consecutive bins, within the 40 ms following stimulus artifact (for details see Belluscio *et al*., 2007). Response latencies were calculated from the beginning of the stimulus artifact to the first bin of the response. Pallidal neurons can show compound excitatory–inhibitory–excitatory responses to cortical stimulation at high stimulation currents (Ryan & Clark, 1991). After examining population PSTHs, which were obtained by averaging the individual PSTHs of all GP neurons responding at 900 μA (see Results), we settled on an upper latency limit of 10 ms for 'early excitations'.

Results

Selection of 6-OHDA-lesioned rats on the basis of performance in the stepping test provided two lesion groups displaying different degrees of impairment in the use of the contralateral forelimb, both differing markedly from control rats (Fig. 1A). The 'moderate deficit' and 'severe deficit' lesion groups made, on average, 70 and 95% fewer steps than control rats, respectively. Post-mortem counts of TH+ neurons in the SNc confirmed that this difference in behavior was associated with different degrees of dopamine neuron loss in the SNc (sham, 98 ± 3%; moderate deficit, 42 ± 6%; severe deficit, 9 ± 9%; remnant TH+ cell bodies ± SEM, relative to the non-lesion side; Fig. 1D and E).

Four to 6 weeks after surgery, we recorded single GP units simultaneously with the motor cortex LFP under urethane anesthesia. Under urethane, the main component of cortical LFP is slow waves (0.5–1 Hz), reflecting synchronous oscillatory activity in a majority of cortical neurons (Steriade, 2000). In our recordings the positive half was the 'active' part of the slow wave (Kasanetz *et al*., 2006). Episodes of cortical 'activation', during which cortical neurons fire

steadily and irregularly, and the cortical LFP is dominated by higher frequency waves of smaller amplitude (Kasanetz *et al*., 2006), occurred spontaneously in most experiments.

Manifest changes in pallidal resting discharge activity occurred only after extensive nigrostriatal degeneration

As reported previously (Magill *et al*., 2001; Goldberg *et al*., 2003; Zold *et al*., 2007), slow modulations in the discharge rate of GP units correlate with cortical slow waves (Fig. 2A). In control rats, pallidal units typically increased their discharge rate during the active part of slow waves (26/31 units in ten rats), with neuronal rhythmic activity lagging behind the cortical LFP by 15 ± 3° at the peak cross-amplitude spectral frequency of 0.88 ± 0.03 Hz (coherence: 0.76 ± 0.03). Confirming our previous findings (Zold *et al*., 2007), 57% of pallidal units of rats having extensive nigrostriatal lesions and severe forelimb use impairment (17/30 in nine rats) showed 'inverse phase' coupling with cortical slow waves, i.e. diminished discharge during peak cortical activity (phase difference of 172 ± 8° at the dominant shared frequency of 0.81 ± 0.03 Hz; coherence, 0.74 ± 0.04; Fig. 2A–C). In the rats that showed moderate forelimb use deficit and partial TH+ neuron depletion, pallidal neurons exhibited the same sort of 'direct phase' correlation with cortical slow waves seen in control rats (22/27 units in ten rats; phase difference, 15 ± 4°; peak frequency, 0.81 ± 0.02 Hz; coherence, 0.74 ± 0.03). Indeed, when the proportion of inverse phase and direct phase neurons was compared between groups, the 'moderate deficit' lesion group differed markedly from the 'severe deficit' lesion group ($P < 0.01$, χ^2 test) but not from control rats (Fig. 2C). In summary, there was no evidence of abnormal cortical slow wave influence on GP firing after partial nigrostriatal damage. However, the lesion produced a conspicuous impairment in forelimb use.

According to a classical model of basal ganglia dysfunction in Parkinson's disease, release of striatopallidal GABAergic neurons from dopamine-induced inhibition should result in a reduction of the average neuronal discharge rate in the GP (Crossman, 1987; Albin *et al*., 1989; DeLong, 1990). However, previous studies do not support this prediction (Filion, 1979; Miller & DeLong, 1988; Pan & Walters, 1988; Filion & Tremblay, 1991; Nini *et al*., 1995; Hutchison *et al*., 1997; Raz *et al*., 2000; Magill *et al*., 2001). Here, notwithstanding the powerful modulation exerted by slow waves in rats with extensive nigrostriatal lesion, the average firing rate of GP neurons was similar in the three experimental groups (Fig. 2D; see also Zold *et al*., 2007). Moreover, we examined GP discharge rates during cortical LFP activation, because pallidal activity may be sensitive to global brain condition (Magill *et al*., 2001), and did not find any difference between groups (Fig. 3A). In addition, average firing rates of 'inverse phase' neurons recorded from 'severe deficit' rats (28.2 ± 1.9 spikes/s, 17 neurons), and 'direct phase' neurons recorded from control rats (28.2 ± 2.1 spikes/s, 26 neurons) and rats of the 'moderate deficit' lesion group (24.7 ± 2.1 spikes/s, 22 neurons), recorded in the slow wave condition, were similar ($F_{2,62} = 0.92$, $P = 0.41$; one-way ANOVA). Therefore, the different degrees of behavioral impairment and TH+ neuron depletion seen in the 'moderate' and 'severe deficit' lesion groups were not associated with changes in average discharge rate in the GP.

Lesion-related changes in resting GP activity, other than modifications of average discharge rate, could have occurred exclusively during cortical LFP activation in the 'moderate deficit' lesion group. As most GP neurons are autonomous pacemakers (see Introduction), we examined discharge regularity during episodes of cortical activation by measuring coefficients of variation and asymmetry in ISIHs (Fig. 3B and Table 1). In control and 'moderate deficit' rats, GP firing was distinctly regular, producing symmetric and sharp ISIHs with small coefficients of variation and asymmetry, while in rats having severe behavioral impairment and extensive nigrostriatal lesion, ISIHs showed a right tail and higher coefficients of variation and asymmetry ($P < 0.01$ vs. either control or 'moderate deficit' rats, Table 1). Importantly, this difference between the 'severe deficit' lesion group and the other experimental groups, in GP discharge pattern during cortical activation, could be completely accounted for by changes occurring in those GP neurons that showed inverse phase activity in the slow wave condition. Indeed, the GP neurons that were classified as 'inverse phase' type during slow wave activity had, during cortical activation, coefficients of variation and asymmetry that were up to 1.6 and 3 times higher, respectively, than those of GP neurons recorded in control and 'moderate deficit' rats (Table 1), and were much more likely to show strings of long ISIs [11 of the 13 neurons that showed inverse phase slow wave activity showed 'pauses' during episodes of cortical activation (Table 2, Fig. 3B)].

To determine if this change in discharge pattern, which selectively affected GP inverse phase neurons, involved an abnormal coupling to cortical activity, we assessed phase locking of GP discharges to the activated cortical LFP, by estimating deviation from uniformity in circular distributions depicting the rate of occurrence of spike discharges at every LFP angle (Fig. 3C). If single unit spiking occurred randomly, instantaneous phases should distribute uniformly. The proportion of GP neurons showing significant phase locking ($P < 0.05$ in the Rayleigh test) was similar in the three experimental groups (5/13 and 5/13, in control and 'moderate deficit' rats, respectively, and 6/13 'inverse phase' GP neurons in 'severe deficit' rats). Moreover, this more irregular firing of GP inverse phase neurons was not associated with novel peaks in cross-amplitude and coherence spectra (data not shown) or with significant spike-triggered averages in cortical LFP waveforms (Fig. 3D). Alternatively, the pauses occurring during cortical activation could be driven by inputs that are not shaped by cortical activity, or result entirely from changes in intrinsic properties of GP neurons. If this were the case, reducing cortical activity dramatically should not prevent the occurrence of pauses. This is difficult to obtain *in vivo*, except for the 'passive' part of slow waves, when cortical neuron activity is almost shut down (Charpier *et al*., 1999; Steriade, 2000; Kasanetz *et al*., 2006). Visual examination of spike trains suggested that 'inverse phase' GP neurons did not pause during the passive part of slow waves (Fig. 2A; see also Fig. 2 in Zold *et al*., 2007). As a means to study regularity of discharge in this condition of reduced cortical drive, we sorted the ISIs of inverse phase GP neurons according to the phase of the ongoing slow wave, selected those ISIs occurring during the passive part (90–270°) of slow waves, and computed their coefficients of variation and asymmetry (Fig. 4). When considering the 11 inverse phase GP neurons that showed pauses during cortical activation, both coefficients were smaller for ISIs occurring during the passive part of slow waves than for ISIs occurring during episodes of cortical activation (0.17 ± 0.006 vs. 0.47 ± 0.06 for coefficient of variation, $P < 0.001$, paired *t*-test; 0.9 ± 0.14 vs. 2.89 ± 0.5 for skewness, $P < 0.01$, paired *t*-test; mean ± SEM, computed after equaling the number of ISIs in both conditions). Moreover, coefficients computed for ISIs occurring during the passive part of slow waves did not differ from estimates obtained from control rat neurons (see Table 1).

In summary, in addition to showing abnormal synchronization with cortical slow waves, GP 'inverse phase' neurons showed an abnormal discharge pattern during cortical activation in rats with

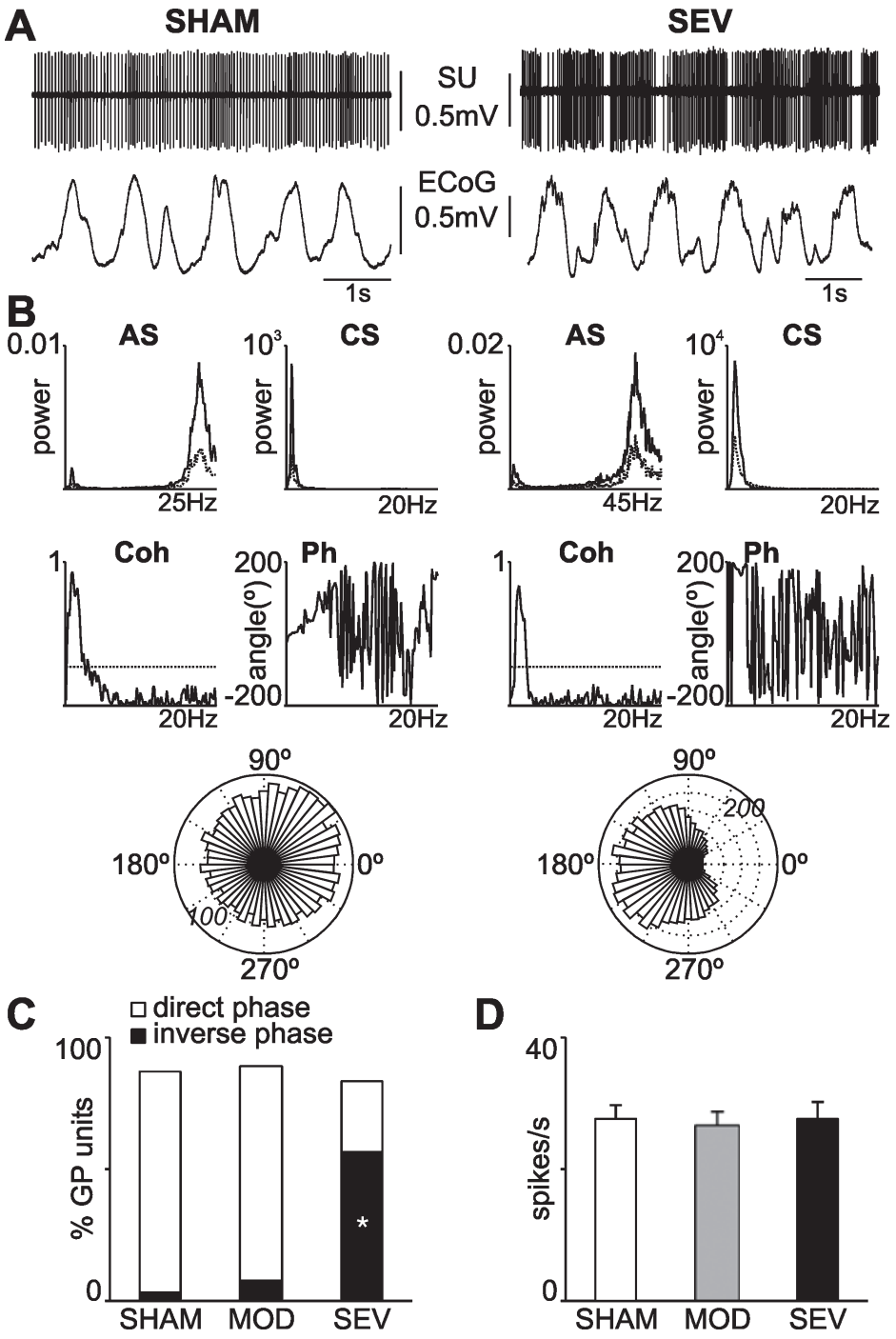

FIG. 2. Resting GP activity during the slow wave state was spared in rats with reliable impairment in forelimb use associated with 58% TH+ neuron depletion in the SNc. The spike trains of most GP units were strongly modulated by cortical slow waves, as described previously, in control and 6-OHDA-lesioned rats. This is here illustrated with (A) representative signal segments and (B) GP neuron power auto-spectra (AS), cross-amplitude spectra (CS), coherence (Coh) and phase spectra (Ph), and circular plots depicting the rate of occurrence of GP spike discharges (radial axis) as function of cortical LFP phase (circular axis). Data are from a 'direct phase' unit recorded from a control rat (left) and an 'inverse phase' unit recorded from a rat with a severe deficit in contralateral forelimb use and fewer than 10% TH+ cells remaining in the SNc (right). Synchronization with cortical slow waves was assessed, over 3-min signal segments, from coherence spectra, at the frequency of the dominant peak (~1 Hz) in cross-amplitude spectra. Dotted lines are confidence limits. In the circular plots, the positive peak of the ECoG is at 0° phase. (C) In control rats and rats with moderate forelimb use impairment, most GP neurons increased their firing rate during the positive part of cortical slow waves, while in rats with severe behavioral impairment, 'inverse phase' coupling prevailed (*$P < 0.01$, χ^2 test, severe deficit lesion group vs. the other groups). (D) The average firing rate (± SEM) of pallidal neurons in the slow wave state was similar in the three experimental groups. All data were obtained from the same rats for which behavioral and neurochemical data are provided in Fig. 1 (31, 27 and 30 GP units from control, 'moderate deficit' and 'severe deficit' lesion groups, respectively).

extensive nigrostriatal lesion and severe forelimb use impairment. This pattern, consisting of brief pauses interrupting tonic discharge, could not be linked to any specific cortical LFP event, but required persistent cortical activity. Moreover, no changes in resting GP discharge activity could be brought to light in the 'moderate deficit' lesion group.

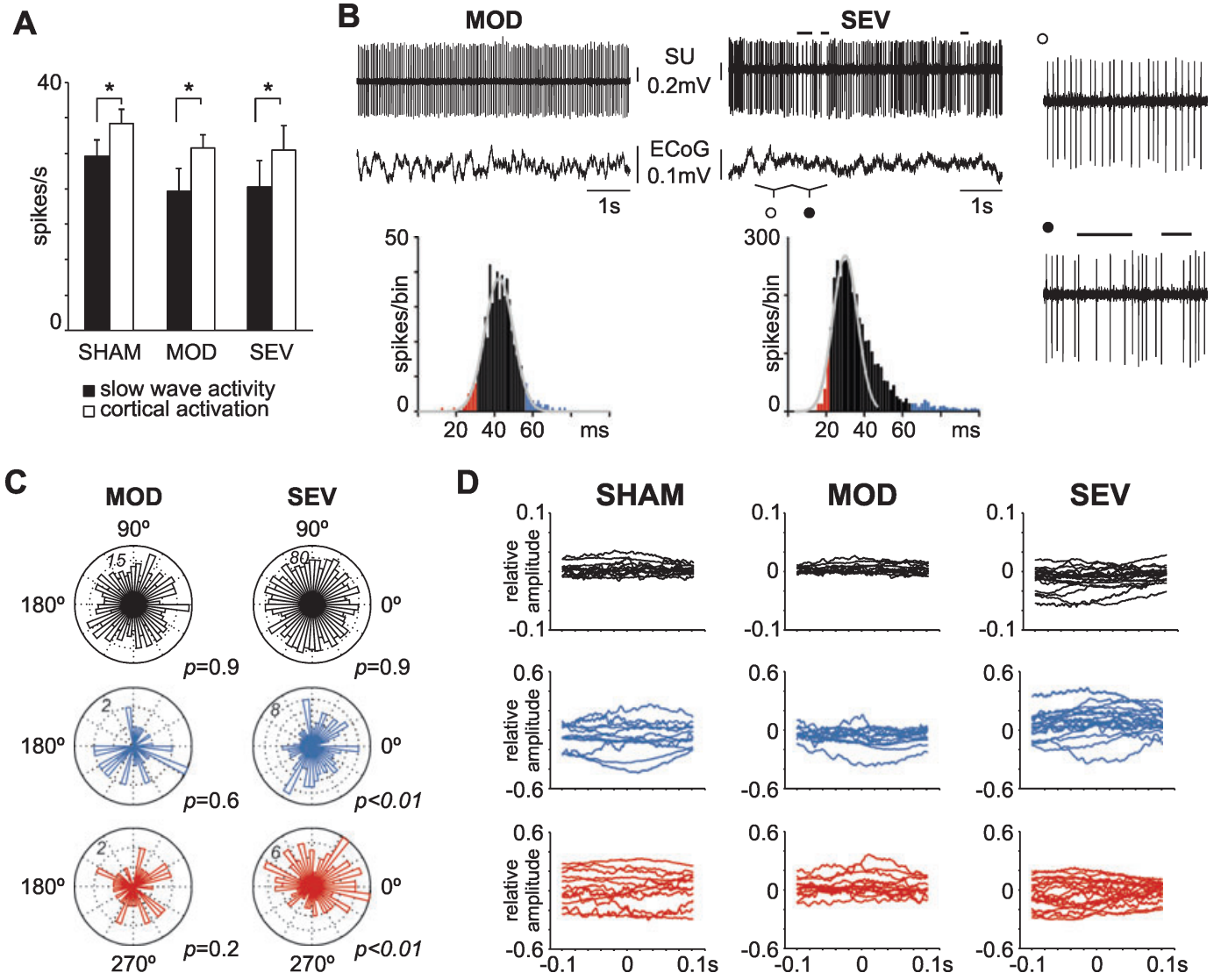

FIG. 3. Resting GP firing pattern was abnormal during episodes of cortical activation in rats with severe nigrostriatal lesion and almost complete forelimb use impairment. The effect of global brain state on GP activity was evaluated in a subset of GP neurons which could be recorded both in the slow wave condition and during episodes of cortical activation lasting 30 s to 2 min (13 neurons from control rats, 13 from 'moderate deficit' rats and 19 from 'severe deficit' rats; among the last, 13 were inverse phase neurons in the slow wave condition). (A) During episodes of cortical activation, the average discharge rate of GP neurons increased (*$P < 0.01$, main effect of brain state, $F_{1,42} = 21.6$), but brain state had no preferential effect on any experimental group (NS main effect of group, $F_{2,42} = 0.9$, and NS interaction, $F_{2,42} = 0.2$, two-way ANOVA with repeated measures on brain state). (B) Despite having similar average discharge rates, during spontaneous episodes of cortical activation, GP neurons had more variable interspike intervals (ISIs) in rats having extensive nigrostriatal damage and severe forelimb use impairment (right) than in rats with moderate behavioral deficit and partial nigrostriatal damage (left). This is illustrated here with representative signal segments (above) and ISI histograms (below). The neuron at right, recorded from a rat with extensive nigrostriatal damage, showed 'inverse phase' activity in the slow wave condition, and pauses in discharge (which are indicated with black lines in the recording) during cortical activation. At right, two 1-s segments of this recording (marked with empty and black circles, respectively) are depicted in more detail to facilitate scrutiny of the pauses. Pauses were detected with a Poisson surprise-based algorithm (see Methods and Table 2). In the ISI histograms the gaussian curve (grey) highlights the right tail in the ISI histogram of the severe deficit lesion group rat. (C) Circular plots depicting the rate of occurrence of GP spike discharges (radial axis) as a function of cortical LFP phase (circular axis), for the above two neurons. In the upper line circular plots (black), all ISIs were used for the computation. Because GP neurons could generate rhythmic activity autonomously, a large part of the ISIs around the mean ISI could be completely unrelated to GP afferent activity. Therefore, we also computed the circular plots for the 5% longest (middle line plots, blue) and 5% shortest (bottom line plots, red) ISIs, which probably held more information about afferent input. Rayleigh *P*-values are provided at the bottom right of each plot. (D) Spike-triggered averages of ECoG activity would reflect synchronization of ISIs to non-oscillatory cortical events occurring during episodes of cortical activation. Here, each graph depicts all the spike-triggered average waveforms computed for sham (13 GP neurons), moderate deficit (13 GP neurons) and severe deficit (19 GP neurons) rats. As in C, spike-triggered averages were computed from all spikes (upper line plots, black), spikes connected with the 5% longest (middle line plots, blue), and spikes connected with the 5% shortest ISIs (bottom line plots, red). None of the triggered waveforms had a significant peak. Overall, there was no significant difference between groups in the proportion of GP neurons modulated by the activated cortical LFP.

Partial nigrostriatal damage induced marked changes in pallidal responsiveness to motor cortex electrical stimulation

Two concentric electrodes placed 1.3 mm apart in the motor cortex (Fig. 1B) allowed us to study, in the GP of these same rats, responsiveness to and spatial selectivity for cortical inputs (Fig. 5). As in control rats, cortical stimulation evoked a complex response in rats with 6-OHDA-induced nigrostriatal lesion, which could comprise an early excitation, an inhibition and a late excitation, at high

TABLE 1. The firing pattern of pallidal neurons was altered during cortical activation in rats with extensive nigrostriatal lesions

	No. of units	CV of ISIs	Coefficient of asymmetry of ISIs	Mean firing rate (spikes/s)	Power of low-frequency ECoG components[§]	
					Slow wave condition	Activated condition
Sham	13	0.22 ± 0.02	0.89 ± 0.3	29.5 ± 3.6	0.88 ± 0.01	0.53 ± 0.04
Moderate deficit lesion group	13	0.24 ± 0.05	0.70 ± 0.2	33.4 ± 4.2	0.88 ± 0.01	0.49 ± 0.04
Severe deficit lesion group	19	0.37 ± 0.04*	2.06 ± 0.4*	29.1 ± 2.6	0.88 ± 0.01	0.51 ± 0.03
Inverse phase neurons in severe deficit lesion group	13	0.43 ± 0.02[†‡]	2.55 ± 0.1[†‡]	28.0 ± 2.1	0.90 ± 0.01	0.53 ± 0.03
Other neurons in severe deficit lesion group	6	0.25 ± 0.02	1.00 ± 0.1	31.4 ± 7.0	0.88 ± 0.02	0.50 ± 0.06

Coefficients of variation and asymmetry of interspike intervals (ISI) of GP neurons recorded during episodes of cortical activation. More than 1000 ISIs per neuron were used for all computations. Data are mean ± SEM. Coefficient of variation = SD/mean ISI. Coefficient of asymmetry= $[n*(\Sigma(x_i - x)^2)]/[(n-1)*(n-2)*SD^3]$; positive values indicate that the distribution is skewed to the right. *The 'severe deficit' lesion group differed significantly from the other two groups (coefficient of variation: one-way ANOVA, $F_{2,42} = 5.77$, $P = 0.006$, $P < 0.05$ vs. either control or 'moderate deficit' rats, Tukey's test; coefficient of asymmetry: one-way ANOVA, $F_{2,42} = 5.37$, $P = 0.008$, $P < 0.05$ vs. either control or 'moderate deficit' rats, Tukey's test). [†]The neurons that showed inverse phase coupling to cortical slow waves in the 'severe deficit' lesion group differed significantly from neurons of the other two groups (coefficient of variation: one-way ANOVA, $F_{2,35} = 13.17$, $P = 5.4 \times 10^{-5}$, $P < 0.05$ vs. either control or 'moderate deficit' rats, Tukey's test; coefficient of asymmetry: one-way ANOVA, $F_{2,35} = 10.39$, $P = 2.8 \times 10^{-4}$, $P < 0.05$ vs. either control or 'moderate deficit' rats, Tukey's test; coefficient of asymmetry: one-way ANOVA, $F_{2,42} = 5.37$, $P = 0.008$, $P < 0.05$ vs. either control or 'moderate deficit' rats, Tukey's test). [‡]Within the 'severe deficit' lesion group, there were significant differences between the neurons that showed inverse phase coupling with cortical slow waves and the other neurons ($P < 0.05$, Student's *t*-test). [§]A repeated-measures two-way ANOVA, performed on the relative power of LFP low-frequency components (0.3–2 Hz), revealed a significant effect of LFP condition ($F_{1,42} = 368.41$, $P = 2.1 \times 10^{-22}$; within-group factor) without effect of experimental group ($F_{2,42} = 0.44$, $P = 0.65$; between-groups factor) and no interaction ($F_{2,42} = 0.48$, $P = 0.62$), indicating that GP recordings were obtained under comparable LFP conditions. All data were obtained from the same rats for which behavioral and neurochemical data are provided in Fig. 1.

TABLE 2. Pallidal neurons were more likely to show pauses during cortical activation in rats with extensive nigrostriatal damage

	No. of units	No. of units with pauses	Pauses per 30 s	Poisson surprise[‡]	Pause duration (s)
Sham	13	3	1.49 ± 0.55	0.29 ± 0.10	0.28 ± 0.06
Moderate deficit lesion group	13	2	0.34//30	0.32//0.31	0.23//0.25
Severe deficit lesion group	19	13*	5.1 ± 1.59	0.33 ± 0.05	0.32 ± 0.05
Inverse phase neurons in severe deficit lesion group	13	11[†]	5.57 ± 1.85	0.35 ± 0.06	0.33 ± 0.06
Other neurons in severe deficit lesion group	6	2	3.5//1.5	0.26//0.17	0.43//0.13

More than 1000 ISIs per neuron were used for all computations. Data are mean ± SEM; where $n = 2$, we provide the value of each neuron. *The 'severe deficit' lesion group differed significantly from the other two groups (Fisher's exact probability test, $P = 0.029$ vs. either control and $P = 0.005$ vs. 'moderate deficit' rats). [†]Within the 'severe deficit' lesion group, the neurons that showed inverse phase coupling to cortical slow waves showed an increased probability of exhibiting pauses in discharge during cortical activation (Fisher's exact probability test, $P = 0.045$ vs. 'other neurons' in severe deficit lesion group). The small number of GP neurons showing pauses in the control and moderate deficit lesion group precluded making statistical comparisons of pause features. Nigrostriatal lesions did not modify burst activity in GP neurons (1/13, 3/13, 4/19 GP neurons with bursts in control, moderate deficit and severe deficit lesion groups, respectively). [‡]A 'Poisson surprise'-based method (Legendy & Salcman, 1985) was used to assess the incidence of pauses and bursts in pallidal neurons recorded during episodes of cortical activation. Surprise equals –log *P*, where *P* is the probability of finding *n* ISIs in a given time interval *T*. *P* is computed under the assumption that firing followed a Poisson distribution, from: $P = e^{-rT} \sum_{i=n}^{\infty} (rT)i/i!$ where *r* is the average discharge rate.

stimulation current (Fig. 5A; see also Ryan & Clark, 1991). Overall, in 6-OHDA-lesioned rats, the GP was markedly over-responsive to motor cortex stimulation when low stimulation currents were used. Forty-four per cent of GP neurons (8/18) responded to stimulation of the medial motor cortex at 300 μA in 'severe deficit' rats, an intensity that did not drive consistent responses in control or 'moderate deficit' rats (0/29 and 3/26, respectively, $P < 0.01$ vs. 'severe deficit' lesion group) (Fig. 5B). At moderate stimulation current (500 μA), GP neurons were more likely to respond to medial motor cortex stimulation in both lesion groups than in control rats. Remarkably, rats of the moderate and severe deficit lesion groups showed similar prevalence of GP responses and both differed markedly from controls (14/26 and 12/18, respectively, vs. 3/29 in controls; $P < 0.01$). At the highest current (900 μA) there were no differences between control (14/29) and 6-OHDA-lesioned rats (18/26 and 13/18, 'moderate' and 'severe deficit' lesion groups, respectively), perhaps because responsiveness in 6-OHDA-lesioned rats had already reached a plateau at 700 μA. As recordings were performed in similar GP regions in the three experimental groups, differences in responsiveness cannot be related to differences in matching between cortical stimulation and GP recording sites (see below). This view is supported by the fact that a subset of the above GP neurons was also over-responsive to lateral motor cortex stimulation in 6-OHDA-lesioned rats (Fig. 5C). Overall, the proportion of GP neurons responding to a single cortical stimulation site in rats belonging to the moderate deficit lesion group was higher than in controls but lower than in rats with extensive nigrostriatal lesion and severe behavioral deficit.

Previous studies have established that pallidal neurons lose selectivity for somatosensory input and sensory-triggered active limb movement in parkinsonian monkeys (Filion *et al*., 1988; Boraud *et al*., 2000; Leblois *et al*., 2006a). To our knowledge, this is the first study

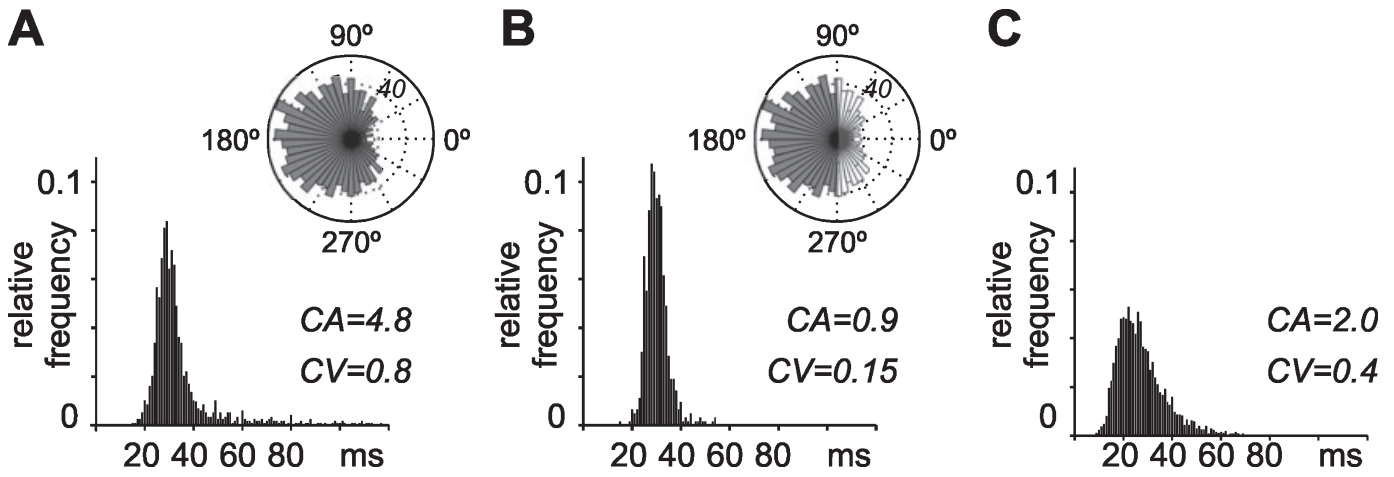

FIG. 4. Discharge activity was regular in 'inverse phase' pallidal neurons when cortical activity was low. To study regularity of discharge in a condition of reduced cortical activity, we estimated the coefficients of variation and asymmetry of interspike intervals (ISIs) occurring during the 'passive' part of slow waves. With this in mind, we selected the 11 GP neurons which showed both inverse phase activity during slow waves and pauses during cortical activation (Table 2). (A) Inter-spike interval histogram (ISIH) of a representative 'inverse phase' GP neuron recorded in the slow wave condition. Inset: circular plot depicting the rate of occurrence of spike discharges (radial axis) as a function of cortical LFP phase (circular axis). (B) Same ISIH, but including only those ISIs that occurred during the 'passive part' of the slow waves (corresponding to angles labeled in black in the inset). (C) For the same GP neuron, frequency distribution of ISIs recorded during an episode of cortical activation. CV, coefficient of variation; CA, coefficient of asymmetry.

comparing the responsiveness of GP neurons to electrical stimulation of two motor cortex sites between rats showing different degrees of motor impairment and SNc lesion. In control rats, few GP neurons responded to both motor cortex sites (1/20 at 500 μA), even at high stimulation current (3/20 at 900 μA; Fig. 5D). In marked contrast, 38% (10/26) and 66% (12/18) of GP neurons responded to both stimulation sites at 500 μA in rats of the 'moderate' and 'severe deficit' lesion groups, respectively ($P < 0.01$). Indeed, the prevalence of convergent responses was similar in both lesion groups at all the stimulation currents tested (14/26 and 12/18 at 900 μA) (Fig. 5D).

Early excitations and inhibitions are believed to result from activation of trans-STN and trans-striatal pathways, respectively (Ryan & Clark, 1991; Kita, 1992; Nambu *et al*., 2000; Kita *et al*., 2004). The late excitation probably involves a rebound excitation of STN neurons (Ryan & Clark, 1991; Kita, 1992; Maurice *et al*., 1998; Bevan *et al*., 2000; Chan *et al*., 2004; Baufreton *et al*., 2005; Kass & Mintz, 2006), but other mechanisms may contribute as well (Kita *et al*., 2004; Leblois *et al*., 2006a). Therefore, we tried to gain further insight into the mechanisms underlying GP over-responsiveness by looking at the different components of the response. At high stimulation current (900 μA), GP neurons mainly showed triphasic excitatory–inhibitory–excitatory responses in the three experimental groups (Figs 5A and 6A). To circumvent a putative ceiling effect, we studied the responses evoked by stimulation of the medial motor cortex at the submaximal stimulation current of 500 μA. Because the proportion of GP neurons responding at this current intensity in control rats was small, in order to have a representative sample, we recorded more GP neurons in additional control rats (35 neurons in eight rats). In rats of the 'severe deficit' lesion group, GP over-responsiveness was mainly due to an increased prevalence of inhibitions and late excitations, which were more common in these rats than in control and moderate deficit lesion group rats (Fig. 6B). Moreover, rats with moderate deficit in contralateral forelimb use and partial nigrostriatal damage exhibited an increased probability of responding with early and late excitations, but not inhibitions, than control rats (Fig. 6B). The small proportion of neurons that could be driven by stimulating electrically a cortical focus in control rats suggests a high degree of functional segregation of cortical inputs within the GP, which deteriorated after nigrostriatal lesion (Fig. 6C).

Discussion

The present results establish for the first time that, in rats showing a conspicuous deficit in forelimb use associated with partial TH+ neuron depletion in the SNc, GP neurons are over-responsive to motor cortex electrical stimulation and lose spatial selectivity for cortical inputs. In addition, we demonstrate for the first time that GP neurons show abnormal resting discharge patterns across different global brain states, without associated changes in average discharge rate, only after massive degeneration of nigrostriatal neurons, in the urethane-anesthetized rat.

Studies performed in rats with unilateral 6-OHDA-induced nigrostriatal lesion have substantially contributed to an understanding of Parkinson's disease pathophysiology (Cenci *et al*., 2002). For instance, rats with severe unilateral 6-OHDA-induced nigrostriatal lesion exhibit a marked deficit in contralateral forelimb use, which can be assessed with the stepping test, and may be analogous to the upper limb akinesia occurring in patients with Parkinson's disease (Olsson *et al*., 1995). Our previous studies in rats showing severe impairment in forelimb use after extensive nigrostriatal lesion have shown that spontaneously hyperactive striatal projection neurons exaggeratedly encode cortical slow rhythms in their spike trains (Tseng *et al*., 2001). More recently, it has been established that the hyperactive striatal neurons project to the GP (Mallet *et al*., 2006). In addition, the resting 'direct phase' hyperactivity of striatopallidal neurons during slow waves is time-coupled to abnormal 'inverse phase' GP firing (Zold *et al*., 2007; see also Walters *et al*., 2007). This evidence pointing to an increased gain of the cortico-striato-pallidal axis is extended by the present finding that GP neurons show an increased probability of responding with an inhibition to motor cortex stimulation in rats with extensive nigrostriatal lesion. Although other mechanisms may contribute to the inhibitory GP responses evoked by cortical stimulation, activation of striatopallidal inhibitory neurons is probably its main source (Ryan & Clark, 1991; Kita, 1992; Kita *et al*., 2004).

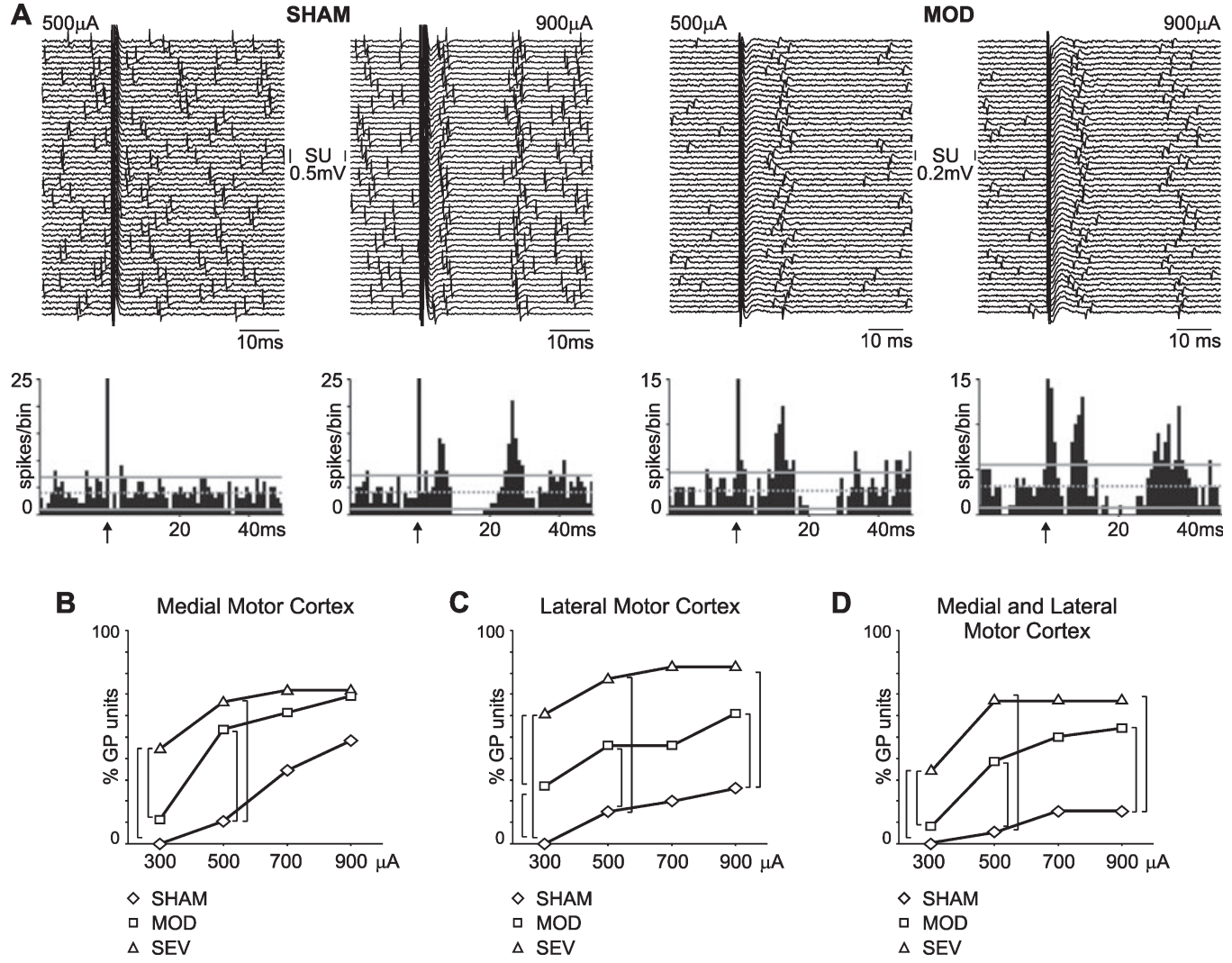

FIG. 5. Increased responsiveness and loss of spatial selectivity for cortical inputs in the GP in rats with moderate behavioral impairment and partial dopamine neuron depletion in the SNc. (A) Peri-stimulus time histograms (PSTHs), built from 100 stimulation trials, illustrating representative responses of GP neurons to motor cortex stimulation at 500 and 900 µA, in a control rat (left) and a rat with a partial nigrostriatal lesion (right). Some of the trials used to compute them are also shown. The dotted lines are the mean ± 75% of the spike per bin counts during the prestimulus period. Arrows indicate stimulus time. (B) The short latency response of GP neurons to stimulation of the medial motor cortex was examined at four stimulation currents, 300, 500, 700 and 900 µA, in 29 units from control rats, 26 units from rats belonging to the moderate deficit lesion group, and 18 units from rats with severe nigrostriatal lesion. Each GP neuron was tested at the four stimulation currents. The graph displays the prevalence of GP neurons showing at least one excitatory or inhibitory response during the 40 ms following the stimuli, at the different currents of stimulation, in the three groups of rats. Points connected by brackets differ by $P < 0.05$, Fisher's exact probability test. (C) In a subset of the above GP neurons, it was possible to study the short latency response to stimulation of a lateral motor cortex site at the four stimulation currents (300, 500, 700 and 900 µA; 20 from control rats, 26 from rats belonging to the moderate deficit group, 18 from rats with severe nigrostriatal lesion). Each GP neuron was tested at the four stimulation currents. Points connected by brackets differ by $P < 0.05$, Fisher's exact probability test. (D) Prevalence of GP neurons responding to both motor cortex stimulation sites, at the four stimulation currents, in the above sample of 20 GP units from control rats, 26 from rats belonging to the moderate deficit group, and 18 from rats with severe nigrostriatal lesion. Points connected by brackets differ by $P < 0.05$, Fisher's exact probability test.

Moreover, after extensive nigrostriatal damage, striatopallidal neurons exhibit increased excitatory responses to motor cortex stimulation (Mallet *et al*., 2006), which could have contributed to increasing inhibitory responsiveness in the GP in our experiments. All the above is in line with the proposal that release of striatopallidal neurons from dopamine-mediated inhibition should result in increased GP inhibition in parkinsonism (Crossman, 1987; Albin *et al*., 1989; DeLong *et al*., 1990). However, when computed over long time-windows, the average discharge rate of GP neurons is not diminished, as predicted by classical basal ganglia models. This suggests that, after extensive nigrostriatal degeneration, the gain of the cortico-striato-pallidal axis increases selectively for highly synchronized cortical inputs (i.e. the active part of slow waves, local transient excitation induced by electrical stimulation).

A remarkable new finding of the present study is that the GP neurons exhibiting abnormal inverse phase coupling with cortical slow waves also show marked changes in resting activity, in the form of pauses interrupting tonic discharge, during episodes of cortical activation. During the cortical activation seen under urethane anesthesia, cortical neurons fire steadily, and rhythmic activity is transient and spatially restricted, resembling what happens in natural awake states and rapid eye-movement sleep (Steriade, 2000). The

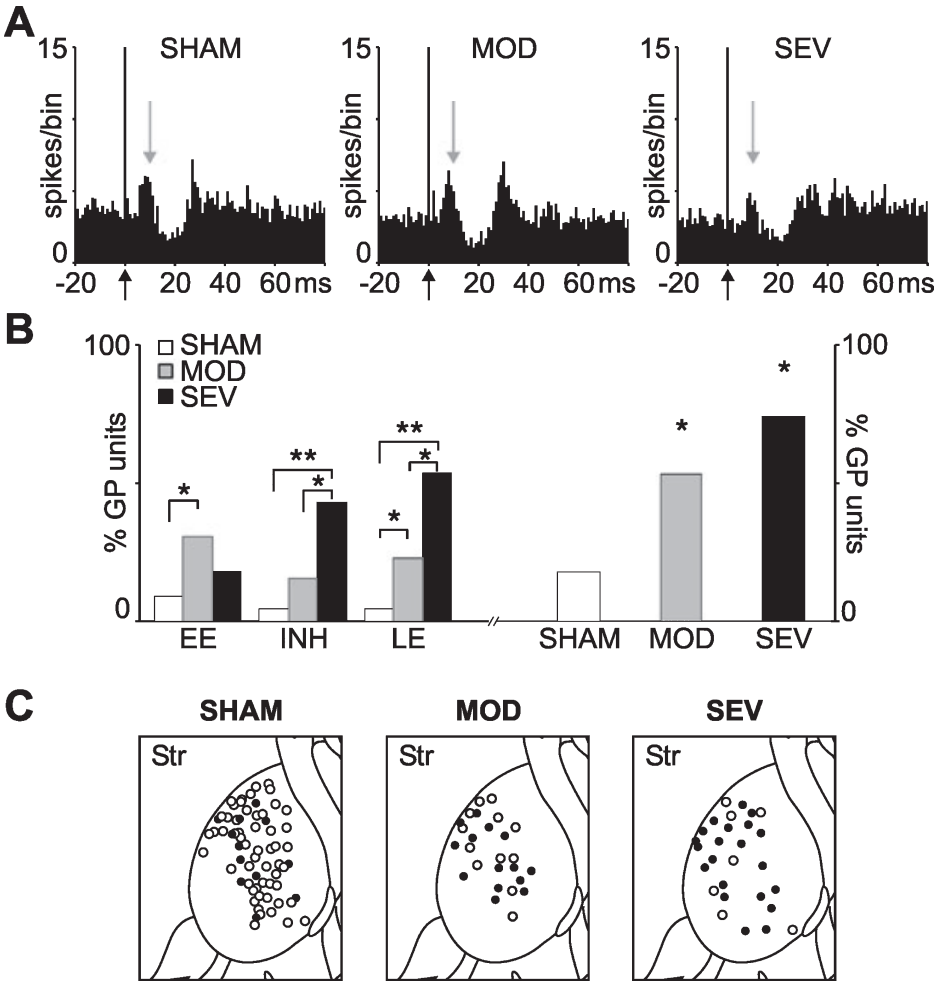

FIG. 6. Increased prevalence of early excitations in rats with partial nigrostriatal damage vs. increased prevalence of inhibitions in rats having extensive nigrostriatal lesions. At the highest stimulation current used in the present study (900 μA), GP neurons showed mostly excitatory–inhibitory–excitatory responses in the three experimental groups. In order to set a latency limit between early and late excitations, we created an 'average PSTH' for each experimental group, by averaging the individual PSTHs of all GP neurons showing a response to medial motor cortex stimulation at 900 μA. (A) The average PSTHs show similar response shapes in the three experimental groups. An upper latency limit of 10 ms (grey arrows) was established for early excitations (mean latency ± SEM: 7.2 ± 0.8, 7.6 ± 0.8, 7.3 ± 0.7 ms for control, moderate and severe deficit rats, respectively, measured from the beginning of the stimulus artifact to the first bin of the response). (B) We compared the prevalence of different components of the short latency response (early excitation – EE, inhibition – INH, late excitation – LE) among the different groups of rats, at a submaximal stimulation current of 500 μA delivered to the medial motor cortex. Because the proportion of GP neurons responding at 500 μA in control rats is small (Fig. 5B), we recorded 35 GP neurons in an additional eight sham lesion rats. In addition, for the present analysis, we included GP neurons that were studied at 500 μA in the original animal groups but could not be studied at higher stimulation currents. This provided samples of 67, 26 and 28 GP neurons for the control, 'moderate' and 'severe deficit' lesion groups, respectively. The bar plot (left part) depicts the proportion of GP neurons showing each component of the response in the three groups of rats. Note that a single neuron could show more than one component, so proportions can add up to more than 100% in a single experimental group. Early excitations were more common in the moderate deficit lesion group than in controls (*$P = 0.019$, Fisher's exact probability test). Inhibitions (INH) were more frequent in the severe deficit lesion group (**$P < 0.0001$ vs. controls, *$P = 0.038$ vs. moderate deficit lesion group), but controls did not differ from moderate deficit rats ($P = 0.09$). Moreover, late excitations (LE) were more common in the moderate deficit lesion group than in controls (*$P = 0.013$), and in the severe than in the moderate deficit lesion group (*$P = 0.028$). In addition, the 'moderate deficit' lesion group showed an overall increased prevalence of GP neurons showing at least one excitatory or inhibitory response component during the 40 ms following the stimuli (right part of the bar plot, *$P < 0.01$ vs. control; see also Fig. 5). (C) Schematic drawings of coronal brain sections (−0.92 mm from bregma; Paxinos & Watson, 1997) showing the distribution of GP neurons responding to medial motor cortex stimulation at 500 μA in the three experimental groups.

fact that the same subset of GP neurons show alterations in resting activity across different global brain states suggests that the mechanisms at play are, to some extent, independent of the pattern of cortical input. This is also suggested by the fact that we could not unveil a distinctive link between abnormal pauses in GP activity and the activated cortical LFP, despite great effort in doing so by seeking for LFP events connected with GP discharges in spike-triggered averages and by computing phase relationships between GP discharges and LFP rhythms. Note that, because many different patterns of synchronized cortical activity may occur along an episode of cortical activation, we could have missed finding a distinctive link between pauses and the activated cortical LFP with methods that presume a stationary association between the signals. Moreover, the span of synchronized activity could be small when the cortex is in the activated condition, so the LFP recording electrode could have missed the cortical activity driving GP pauses. Indeed, as GP neurons fire recurrently in the absence of functional synaptic input, it is usually assumed that the pauses they show *in vivo* are caused by synaptic inhibition (Nambu & Llinas, 1994; Cooper & Stanford, 2000; Chan *et al*., 2004). In line with this view, pauses did not occur

during the 'passive' part of slow waves, when most cortical neurons are silent (Charpier *et al*., 1999; Steriade, 2000; Kasanetz *et al*., 2006), but only when substantial cortical neuronal activity was present (cortical activation, active part of slow waves). This result does indicate that cortical drive modulation of GP–STN network activity is necessary for pauses to occur.

Competition between movement-promoting and movement-arresting trans-basal ganglia pathways may underlie action selection and movement sequencing (Chevalier & Deniau, 1990; Mink, 1996; Redgrave *et al*., 1999; Hikosaka *et al*., 2000). Consequently, an increased gain of the movement-arresting pathways may contribute to impeding voluntary movement in Parkinson's disease (Albin *et al*., 1989; DeLong, 1990; Mink, 1996; Boraud *et al*., 2000). In normal conditions, pauses in GP neuronal activity may contribute to action selection and sequencing by releasing discharge activity downstream in the STN and basal ganglia output nuclei, and ultimately causing inhibition of thalamic motor nuclei and suppressing non-preferred actions (Mink, 1996; Turner & Anderson, 1997; Hikosaka *et al*., 2000). In this context, an increased trend of GP neurons to pause, as seen here after extensive nigrostriatal degeneration, may contribute to arresting movement in advanced Parkinson's disease. As a consonance, in monkeys, abrupt inhibition of external globus pallidus activity by local injection of the $GABA_A$ agonist muscimol results in a flexed arm posture and co-contraction of agonist and antagonist muscles (Kato & Kimura, 1992), whereas in rats, muscimol-induced GP inhibition causes catalepsy (Matsui & Kamioka, 1978).

Remarkably, rats showing a marked (though not complete) impairment of forelimb use associated with ~58% TH+ neuronal loss in the SNc did not show an increased prevalence of 'pausing' neurons in the GP, nor any other noticeable change in resting GP discharge activity across cortical activity states. This finding is consistent with predictions by a recent computational modeling study suggesting that basal ganglia neurons would not show abnormal resting activity unless extensive dopamine depletion is produced (Leblois *et al*., 2006b). However, this model points to an increased gain of a loop involving the cortico-STN pathway as the main cause of changes in resting discharge activity, while our present and previous (Zold *et al*., 2007) findings suggest an involvement of inhibitory input conveyed by the cortico-striato-pallidal axis. Both studies converge on the view that changes in resting discharge activity may be relevant to an understanding of clinical features of advanced Parkinson's disease, although other mechanisms may underlie early motor impairment.

Pallidal neurons showed a three times higher probability of responding, mainly with excitations, to stimulation of a cortical locus, and accordingly, the GP lost spatial selectivity for cortical inputs in rats with partial nigrostriatal damage. It has been established that STN lesions (Ryan & Clark, 1991; Kita, 1992), $GABA_A$ agonist injections in the STN (Nambu *et al*., 2000), or blockade of glutamate receptors in the GP or STN (Nambu *et al*., 2000; Kita *et al*., 2004) eliminate the excitatory GP responses to cortical stimulation and make the inhibition more prominent. Because the inhibitory and excitatory responses have partially overlapping time courses (Kita, 1992), reduced inhibition could account for increased excitation. Striatopallidal neurons are hyperresponsive to cortical stimulation after extensive nigrostriatal damage (Mallet *et al*., 2006), so reduced striatal inhibition is unlikely after partial lesions. The other putative source of inhibition is local axon collaterals of GP neurons, which could be activated during the early excitation (Kita *et al*., 2004; Paz *et al*., 2005). Computational modeling suggests that reduced intrapallidal inhibition promotes local synchronization (Terman *et al*., 2002). However, the spontaneous IPSCs shown by GP neurons *in vitro*, which are presumably driven by local axon collaterals, are not modulated by dopamine (Cooper & Stanford, 2001). Moreover, there is no empirical evidence to support that GP neurons undergo changes in intrinsic membrane mechanisms in parkinsonism. Alternatively, enhanced excitatory responsiveness could be related to an increased gain of the cortico-STN pathway. Subthalamic nucleus resting hyperactivity is well documented in animal models of advanced Parkinson's disease (for reviews see DeLong, 1990; Hirsch *et al*., 2000; Bevan *et al*., 2002). Moreover, a time-course study of STN resting activity after 6-OHDA-induced nigrostriatal lesions demonstrated that STN hyperactivity occurs early during the progression of nigrostriatal degeneration (Vila *et al*., 2000). We speculate that the trans-STN pathway's gain increase is higher, after partial nigrostriatal degeneration, than that of the trans-striatopallidal pathway, but the latter pathway would become dominant after extensive damage. The fact that striatopallidal inhibition partially overlaps early excitation (Kita, 1992) might explain why early excitations were less frequent after extensive than partial nigrostriatal damage. This working hypothesis could explain why late excitations, instead of receding, became more prominent after extensive nigrostriatal degeneration, as disinhibition boosts STN activity and contributes to late excitations (Maurice *et al*., 1998; Bevan *et al*., 2000; Chan *et al*., 2004; Baufreton *et al*., 2005; Kass & Mintz, 2006). However, studies of STN and striatal responsiveness to cortical stimulation after partial nigrostriatal damage are lacking, so the mechanisms underlying altered GP responsiveness remain speculative.

Functional segregation of information processing within the direct trans-striatal pathway is an essential feature of basal ganglia operation (Alexander *et al*., 1986; Chevalier & Deniau, 1990), and loss of spatial segregation capacity of sensory- and movement-related activity in the basal ganglia output nuclei may impede action selection (Filion *et al*., 1988; Boraud *et al*., 2000; Leblois *et al*., 2006a). However, no study has yet been able to dissociate dopamine depletion-induced changes in sensorimotor-related activity from changes in resting activity. It is usually assumed that the trans-STN and trans-striatopallidal pathways carry a relatively non-specific movement-arresting signal that diffusely excites basal ganglia output nuclei neurons, while focussed inhibition by the direct pathway facilitates movement (Mink, 1996; Hikosaka *et al*., 2000). However, neuronal activity during behavioral tasks is at least as complex in the GP as in the basal ganglia output nuclei (Mink & Thach, 1991; Turner & Anderson, 1997, 2005), to the extent that GP neurons may independently and dynamically process movement parameters and reward probability (Arkadir *et al*., 2004). Importantly, most pallidal neurons innervate, in addition to the STN, basal ganglia output nuclei neurons and striatal GABA interneurons (Bolam *et al*., 2000). A loss of spatial discrimination and an abnormal temporal integration of cortical inputs at the level of individual GP neurons, occurring independently of changes in resting activity after partial nigrostriatal lesion, may contribute to the early motor impairment in Parkinson's disease, not only by driving abnormal activity downstream in the STN and basal ganglia output nuclei, but also by modifying the flow of information through the striatum (Mallet *et al*., 2006).

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Abbreviations

6-OHDA, 6-hydroxydopamine; GP, globus pallidus; ISI, interspike interval; ISIH, interspike interval histogram; LFP, local field potential; PSTH, peristimulus time histograms; PV, parvalbumin; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

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