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Consequences of partial and severe dopaminergic lesion on basal ganglia oscillatory activity and akinesia

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Abstract

Severe chronic dopamine (DA) depletion increases the proportion of neurons in the basal ganglia that fire rhythmic bursts of action potential (LFO units) synchronously with the cortical oscillations. Here we report on how different levels of mesencephalic DA denervation affect substantia nigra pars reticulata (SNpr) neuronal activity in the rat and its relationship to akinesia (stepping test). Chronic nigrostriatal lesion induced with 0 (control group), 4, 6 or 8 μg of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle resulted in a dose-dependent decrease of tyrosine hydroxylase positive (TH^+) neurons in the SN and ventral tegmental area (VTA). Although 4 μg of 6-OHDA reduced the number of TH^+ neurons in the SN by ~60%, both stepping test performance and SNpr neuronal activity remained indistinguishable from control animals. By contrast, animals that received 6 μg of 6-OHDA showed a marked reduction of TH^+ cells in the SN (~75%) and VTA (~55%), a significant stepping test deficit and an increased proportion of LFO units. These changes were not dramatically enhanced with 8 μg 6-OHDA, a dose that induced an extensive DA lesion (> 95%) in the SN and ~70% reduction of DA neurons in the VTA. These results suggest a threshold level of DA denervation for both the appearance of motor deficits and LFO units. Thus, the presence of LFO activity in the SNpr is not related to a complete nigrostriatal DA neuron depletion (ultimate stage parkinsonism); instead, it may reflect a functional disruption of cortico-basal ganglia dynamics associated with clinically relevant stages of the disease.

Introduction

In vivo electrophysiological studies performed in animal models of Parkinson's disease and parkinsonian patients indicate that chronic dopamine (DA) depletion alters significantly the discharge activity of basal ganglia neurons (Boraud *et al*., 2002; Murer *et al*., 2002; Brown, 2003). The emergence of cells firing bursts of action potentials (Sanderson *et al*., 1986; MacLeod *et al*., 1990; Filion & Tremblay, 1991; Murer *et al*., 1997; Boraud *et al*., 1998; Tseng *et al*., 2000, 2001a) and showing periodic oscillations in firing rate (Nini *et al*., 1995; Wichmann *et al*., 1999; Raz *et al*., 2000; Tseng *et al*., 2001a,b) were repeatedly observed in chronic DA-depleted brains. Although the mechanisms that trigger and maintain these changes are not completely understood, it is believed that the presence of an abnormal activity in the basal ganglia may underlie some of the clinical manifestations observed in Parkinson's disease (Boraud *et al*., 2002; Murer *et al*., 2002; Brown, 2003).

Recordings performed in 6-hydroxydopamine (6-OHDA)-lesioned rats have revealed an increased proportion of neurons displaying low-frequency oscillatory activity (LFO, 0.5–2 Hz) in their firing rate characterized by rhythmic bursts of action potentials (Magill *et al*., 2001; Tseng *et al*., 2001a,b; Belluscio *et al*., 2003). For example, 40–50% of neurons recorded in the substantia nigra pars reticulata (SNpr; the main basal ganglia output nuclei in rodents) of 6-OHDA animals were recognized as LFO units (Tseng *et al*., 2001a) firing bursts of action potentials synchronously with the cortical rhythms (Belluscio *et al*., 2003). This bursting activity is strongly modulated by striatal DA receptors and the subthalamic nucleus (STN): (i) striatal stimulation of D1 receptors suppressed the LFO pattern in the SNpr of 6-OHDA rats (Tseng *et al*., 2000); (ii) the proportion of SNpr LFO units was significantly reduced in 6-OHDA animals with an STN lesion (Murer *et al*., 1997; Tseng *et al*., 2000, 2001a). These results suggest that chronic DA depletion may promote the spreading of cortical rhythms to the basal ganglia output nuclei through the DA-depleted striatum and STN (Murer *et al*., 2002). However, it remains unclear whether the appearance of LFO activity in the basal ganglia results from a severe DA depletion (as observed in advanced parkinsonism) or it reflects a functional disruption of cortico-basal ganglia dynamics that may be linked to earlier stages of the parkinsonian condition. To address this issue, we investigated the impact of different levels of mesencephalic DA denervation on SNpr neuronal activity and its relationship to akinesia. SNpr single unit activity was recorded in urethane-anaesthetized rats that received a single unilateral injection of vehicle, 4, 6 or 8 μg of 6-OHDA in the medial forebrain bundle. The degree of motor impairment was determined by the stepping test before assessing the electrophysiological recordings, and the extent of DA denervation was estimated post-mortem by counting the number of tyrosine-hydroxylase positive (TH^+) neurons in the substantia nigra (SN) and the ventral tegmental area (VTA).

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Materials and methods

All experimental procedures were performed according to local regulations regarding the care and use of laboratory animals (Servicio Nacional de Sanidad y Calidad Agroalimentaria, RS 617/2002, Argentina). All animals used in the present study were maintained on a 12 : 12-h light/dark cycle, with food and tap water available *ad libitum*, until the time of the experiment.

6-OHDA lesions and stepping test

Male adult Sprague–Dawley rats weighing 190–220 g were randomly assigned to receive the neurotoxin 6-hydroxydopamine hydrobromide (Sigma) or a sham lesion. As reported previously (Tseng *et al*., 2000, 2001b), the rats were anaesthetized with pentobarbital (50 mg/kg i.p.) and a single stereotaxic injection of 6-OHDA was delivered into the left medial forebrain bundle (stereotaxic coordinates A: −4.3 from bregma, L: 1.6, H: −8.3 below the cortical surface, Paxinos & Watson, 1997). To induce different degrees of DA denervation, three doses of 6-OHDA were chosen: 4, 6 and 8 μg of 6-OHDA free base in 4 μL of 0.1% ascorbic acid (Sigma). The sham lesion was performed following the same experimental procedure, but with the vehicle solution. The injection rate was 0.4 μL/min and the cannula was left in place for an additional 2 min before slowly being removed.

To examine the effect of 6-OHDA on forelimb akinesia, the performance of each animal was measured using the stepping test by an investigator blind to the animal's status (Olsson *et al*., 1995). Two weeks after surgery, the animals were handled during the subsequent 3–4 days to become familiar with the experimenter's grip. The stepping test was conducted twice per day for three consecutive days by the same experimenter and recorded on videotapes for offline analysis. For each session, the rat was held in one hand fixing the hindlimbs whereas one of the forelimbs was slightly fixed with the other hand. In this position and with the other forepaw touching the surface of the table, the rat was moved, first to the forehand and then to the backhand direction. Forward adjusting steps were defined as the movement of the forepaw toward the torso to compensate for the outward lateral movements of the body. Backward adjusting steps were defined as the movement away from the torso to compensate for the inward medial movement of the body. The number of adjusting steps was counted in both directions for each forelimb and a mean value was obtained by averaging the number of steps observed across the six sessions.

Extracellular recordings and electrophysiological data analysis

All electrophysiological recordings were conducted between 6 and 8 weeks after the lesion. Rats were anaesthetized with urethane (1.2 g/kg, i.p.), treated with a local anaesthetic (lidocaine) on the scalp and pressure points, and secured to the stereotaxic frame (Stoelting, Wood Dale, IL, USA), as previously reported (Tseng *et al*., 2001b). Briefly, rat temperature was maintained at about 37–38 °C with a heating pad (custom made), and additional urethane (usually, supplements of 0.4 g/kg s.c. every 3–4 h; Tseng *et al*., 2001b) was administered throughout the experiment as necessary in order to maintain a constant level of anaesthesia, as determined by testing the hindlimb withdrawal reflex, and electrocorticographic recordings when available. Glass microelectrodes with tip diameters of 2–5 μm (1–10 MΩ) and filled with 1% Pontamine Sky Blue in 2 M NaCl were placed in the SNpr at the following coordinates: 5.3–5.8 mm posterior from bregma, 1.6–2.4 mm lateral from the middle line, and 7.5–8.6 mm below the cortical surface (Paxinos & Watson, 1997). The electrodes were hydraulically advanced through the SNpr until single units could be isolated. The isolated units were monitored for at least 10 min to ensure the stability of their firing rate, firing pattern and spike morphology, and then 3–5 min of spontaneous activity were recorded. The signal was filtered (bandwidth 300–3000 Hz), amplified (ER-98, NeuroData, Delaware Water Gap, PA, USA), sent to an A/D converter (DigiData 1200, Axon Instruments, Foster City, CA, USA) and acquired with Axoscope 1.1 (Axon Instruments) at a sampling rate of 10 kHz. In some experiments a concentric bipolar electrode (SNE-100, Better Hospital Equipment, New York, USA) was placed in the frontal cortex (3 mm anterior to bregma, 1.5 mm lateral, 1.5 mm below the cortical surface; Paxinos & Watson, 1997) to obtain a differential recording of the electrocorticogram (ECoG; filtered at 0.1–300 Hz) simultaneously with SNpr single-unit activity. At the end of the experiment the tip position of the glass electrode was marked by an iontophoretic deposit of Pontamine Sky Blue and the rat was transcardially perfused with cold saline (150–200 mL) followed by 4% paraformaldehyde (4% PFA, 200 mL) in phosphate-buffered saline (PBS). The brain was removed, incubated overnight in 4% PFA and stored in PBS containing 15% sucrose for 3–5 days.

All time series analyses were performed using Statistica 4.2 (Statsoft Inc., Tulsa, OK, USA), and the interspike interval (ISI) of the signal was obtained by means of spike amplitude discrimination. Autocorrelograms of the ISI were computed from 3-min segments of signal, using 3-ms bins over 1000 bins (a total lag of 10 s). The autocorrelograms were smoothed using a moving average method, and then subjected to a fast Fourier transform (FFT), yielding power spectra with 0.1-Hz resolutions. Both the autocorrelograms and the spectral analysis were used to characterize the periodic components in the spike trains of the recorded unit. As reported previously (Tseng *et al*., 2001a), spectral densities were obtained using a Hanning window (width = 5) and the relative power was calculated by normalization to the total power within the frequency range of 0.017–66.6 Hz (the power of frequencies above 10 Hz was negligible). Peaks exceeding the 95% confidence interval of the mean relative spectral power were considered significant (Tseng *et al*., 2001a).

The 'degree of burstiness' was estimated by measuring the number of bursts/3000 spikes (NB) and the Poisson surprise (PS) following an algorithm described by Legéndy & Salcman (1985). As described previously (Tseng *et al*., 2000, 2001a), bursts were defined as at least three consecutive ISIs with a duration shorter than half that of the mean ISI of 3000 spikes. The PS of a burst is a measure of how improbable it is to find, by chance, a similar sequence of ISIs in a neuron with a random (Poisson) spike discharge pattern.

In some experiments, we performed simultaneous recordings of frontal cortex ECoG and SNpr single-unit activity. ECoG sampling was reduced to 1000 Hz and cross-correlograms of the signals were computed for delays of 3 s with a resolution of 3 ms. Peaks exceeding three correlated white noise standard errors were considered significant. The similarity in the oscillatory frequency content of the signals was further investigated using spectral analysis and coherence estimation (Lopes da Silva *et al*., 1989; Tseng *et al*., 2001b). Power spectra and coherence were computed as described in Tseng *et al*. (2001b) (resolution for 30-s signal segments of 0.033 Hz). Coherence attains its highest value when the phase shift and ratio between the amplitudes of the two waveforms remains constant. A significant coherence (> 0.6) in at least three disjoined 30-s signal segments at the dominant frequency of the cross-spectra was considered as an indication of a high probability of synchronization (Tseng *et al*., 2001b).

Tissue processing and immunohistochemistry

All animals were transcardially perfused with cold saline (150–200 ml) followed by 4% paraformaldehyde (4% PFA, 200 ml) in phosphate buffered saline (PBS) as describe elsewhere (Tseng *et al*., 2001b). The brain was removed, incubated overnight in 4% PFA, and stored in PBS containing 15% sucrose for 3–5 days. Serial coronal sections 40 μm thick were obtained from the midbrain containing the SN and VTA. Most of these sections were Nissl stained and used to reconstruct the recording sites (Murer *et al*., 1997; Tseng *et al*., 2000), the exact position of which varied across animals. The extent of dopaminergic lesion was estimated by means of TH immunohistochemistry performed on free-floating sections as previously reported (Murer *et al*., 1997). All sections included for cell counting were taken about 1 mm from the site of 6-OHDA injection. High-resolution images of the TH staining were captured with a digital camera (Nikon) connected to a microscope (Olympus) and then transferred to a computer for analysis. Only TH-immunoreactive images containing a visible nucleus and at least one emerging dendritic process were considered as TH^+ cells. The anatomical limit between SN and VTA was defined by a vertical line passing through the medial border of the cerebral peduncle (Kirik *et al*., 2001). Both SN and VTA TH^+ neurons were counted at four coronal sections per animal between stereotaxic planes 5.3–6.0 mm posterior from bregma by an experimentally blinded investigator using the ImageJ software (National Institute of Health, USA, http://rsb.info.nih.gov/ij/). For statistical comparisons, single SN and VTA values per animal were obtained by averaging the number of remaining TH^+ cells observed in these four sections.

Statistical analysis

Differences among experimental conditions were considered statistically significant at $P < 0.05$. The Fisher exact probability test (FET) was used to examine the relationship between two dichotomous variables and Student's *t*-test was applied for two-group comparisons involving a single continuous variable. Repeated-measures ANOVA was used to compare the effect along two or more variables.

Results

All sham and lesioned animals were included in this study. Chronic nigrostriatal lesion induced by single unilateral injection of 6-OHDA into the medial forebrain bundle resulted in a dose-dependent decrease of mesencephalic TH^+ neurons. A mean number of SN and VTA TH^+ cells (cells per section) was obtained per animal by averaging the number of remaining TH^+ neurons observed in four coronal sections (see Materials and methods). As summarized in Fig. 1, no apparent effect was observed in the SN (ipsilateral: 149.8 ± 11.1 cells per section; contralateral: 158.2 ± 14.7 cells per section; mean ± SEM) or the VTA (ipsilateral: 192.6 ± 31.9; contralateral: 191 ± 26.8) of vehicle-treated animals ($n = 6$). Injection of 4 μg 6-OHDA ($n = 8$) decreased significantly the number of TH^+ neurons in the SN (ipsilateral: 77 ± 7.2 cells per section; contralateral: 158.3 ± 6.9 cells per section; $P < 0.0001$ compared with vehicle, Tukey *post-hoc* test after significant ANOVA) without affecting extensively the VTA (ipsilateral: 187.4 ± 15.8 cells per section; contralateral: 211.8 ± 18.3 cells per section). By contrast, a marked reduction of TH^+ cells was observed in both the SN (ipsilateral: 33.83 ± 5.1 cells per section; contralateral: 151.7 ± 11.2 cells per section; $P < 0.0001$ compared with vehicle or 4 μg 6-OHDA groups) and the VTA (ipsilateral: 90.2 ± 4.9 cells per section; contralateral: 198.8 ± 9.6 cells per section; $P < 0.005$ compared with vehicle or 4 μg 6-OHDA groups) of 6 μg 6-OHDA-lesioned rats ($n = 6$). The extent of these lesions was significantly pronounced following 8 μg of 6-OHDA ($n = 6$), resulting in an almost complete DA neuron depletion in the SN (ipsilateral: 7.3 ± 1 cells per section; contralateral: 158.2 ± 5.2 cells per section; $P < 0.0001$ compared with vehicle, 4 μg or 6 μg 6-OHDA groups) and ∼70% reduction of TH^+ neurons in the VTA (ipsilateral: 63.2 ± 8.2 cells per section; contralateral: 210.2 ± 14.3 cells per section; $P < 0.0001$ compared with vehicle or 4 μg 6-OHDA groups, and $P < 0.03$ compared with 6 μg 6-OHDA group). These results indicate that single injection of 6-OHDA into the medial forebrain bundle induced a relatively higher degree of DA cell lesion in the SN than in the VTA, even though both regions were dose-dependently affected (Fig. 1).

The effect of different degrees of DA denervation on forelimb akinesia was examined by assessing the stepping test (Olsson *et al*., 1995) 2 weeks after the lesion. An additional stepping test was conducted just before the electrophysiological recordings (6–8 weeks after the lesion). Because no apparent differences were observed among these sessions, the data were pooled. As expected, forelimb stepping ability (measured as the number of adjusting steps) was not affected in the vehicle group as compared with untreated/normal rat's behaviour (data not shown). Both the untreated and the vehicle-treated animals (Fig. 2A) showed almost identical symmetric stepping performance. A significant reduction of adjusting steps was observed in the contralateral forelimb of lesioned rats (as referred to the lesioned side), but only following 6 or 8 μg of 6-OHDA (Fig. 2A). Animals that received 4 μg of 6-OHDA showed a symmetric stepping performance indistinguishable from that of the vehicle group (Fig. 2A), whereas 6 and 8 μg 6-OHDA-lesioned rats showed similar asymmetric stepping deficits (Fig. 2A), even though they exhibited different levels of DA lesion (see above, Fig. 1). As summarized in Fig. 2B, only animals that had a DA lesion > 65% in the SN displayed marked changes in the number of adjusting steps (e.g. contralateral forelimb). These results are consistent with previous reports (Kirik *et al*., 1998; Chang *et al*., 1999) showing that a threshold of DA cells loss is required to produce deficits in the stepping test.

In vivo recordings of SNpr single-unit activity were conducted in urethane-anaesthetized animals from all experimental groups described above. All units included in the present study were recorded among 5.3–5.8 mm posterior from bregma, 1.6–2.4 mm lateral from the middle line and 7.5–8.6 mm below the cortical surface (Paxinos & Watson, 1997). According to our previous reports (Murer *et al*., 1997; Tseng *et al*., 2001a; Belluscio *et al*., 2003), > 90% of neurons (35/38) in the SNpr of vehicle-treated animals displayed a regular/tonic firing pattern (Fig. 3A) whereas the remaining three neurons were recognized as LFO units (Fig. 3B). Similar mean firing rate (vehicle: 24.17 ± 2.37 Hz vs. 4 μg 6-OHDA: 23.36 ± 2.48 Hz) and proportion of non-LFO units (46/49) were observed in the SNpr of 4 μg 6-OHDA-lesioned rats (Fig. 3C). By contrast, SNpr neurons in 6 and 8 μg 6-OHDA-lesioned rats showed a significant decrease of mean firing rate (6 μg 6-OHDA: 14.74 ± 1.43 Hz, $n = 48$, $P < 0.03$ compared with vehicle or 4 μg 6-OHDA; 8 μg 6-OHDA: 14.88 ± 2.03 Hz, $n = 46$, $P < 0.04$ compared with vehicle or 4 μg 6-OHDA; Tukey *post-hoc* test after significant ANOVA) and a higher proportion of LFO units (6 μg 6-OHDA: 13/48, $P = 0.0273$ compared with vehicle and $P = 0.0063$ compared with 4 μg 6-OHDA; 8 μg 6-OHDA: 18/46, $P = 0.001$ compared with vehicle and $P = 0.0001$ compared with 4 μg 6-OHDA; FET) (Fig. 3). Power spectra analysis showed similar dominant peak frequencies among SNpr LFO units recorded from 6 and 8 μg 6-OHDA rats (0.74 ± 0.09 Hz vs. 0.78 ± 0.11 Hz, respectively). These LFO units

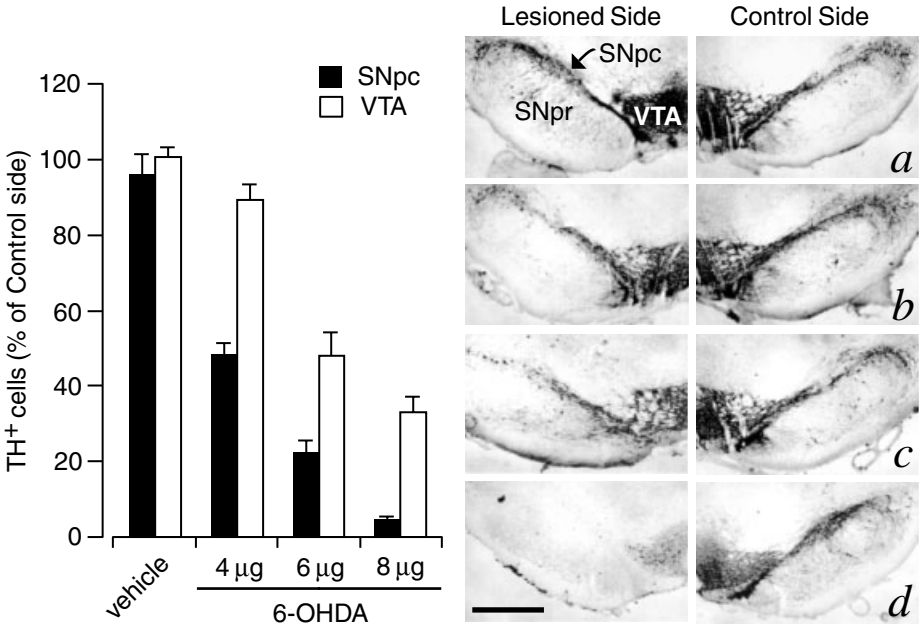

FIG. 1. Chronic DA nigrostriatal lesion induced by increasing doses of 6-OHDA. Left: bar graph (mean ± SEM) summarizing the dose-dependent effect of 6-OHDA (vehicle, 4, 6 and 8 µg) on the number of TH^+ neurons (indicated as percentage of control side) in the SN and VTA. A dose-dependent decrease of TH^+ neurons was observed in both structures. Right: representative images of TH immunostaining showing the degree of DA denervation observed in the SN and VTA of animals that received vehicle ($n = 6$) (a), 4 µg ($n = 8$) (b), 6 µg ($n = 6$) (c) or 8 µg ($n = 6$) (d) of 6-OHDA. Scale bar, 0.7 mm.

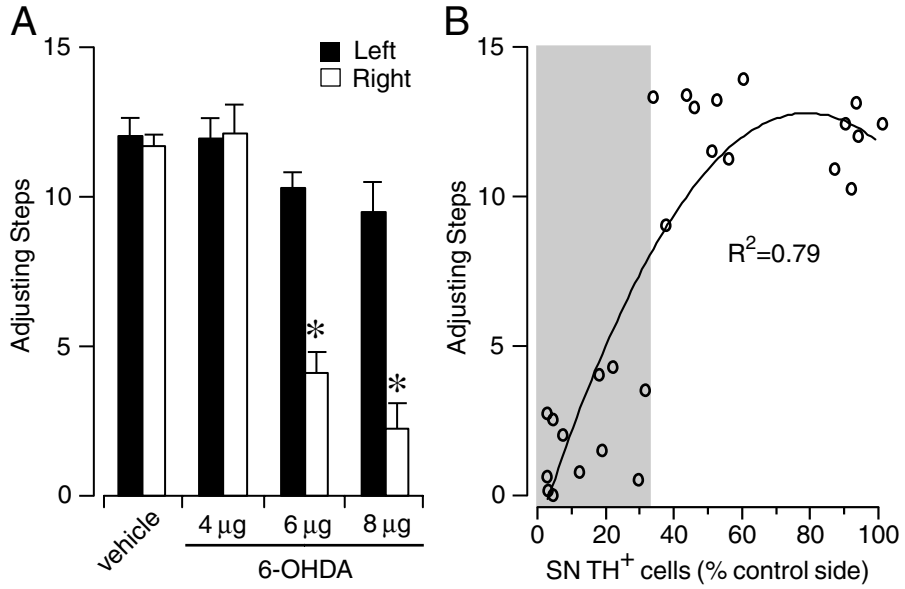

FIG. 2. Motor deficits induced by increasing doses of 6-OHDA. (A) Bar graph summarizing the effect of 6-OHDA on the stepping test performance. Forelimb adjusting steps ability was not affected in vehicle or 4 µg 6-OHDA-treated animals. A significant decrease of adjusting steps was observed in the contralateral (right) forelimbs only in animals that received 6 µg and 8 µg of 6-OHDA (*$P < 0.01$, Tukey *post-hoc* test after significant ANOVA). (B) Scatter plot illustrating the relationship between the level of DA neuron depletion in the SN (indicated as TH^+ cells) and the contralateral forelimb stepping test performance (indicated as the number of adjusting steps). Stepping test deficit does not correlate linearly with the level of DA denervation in the SN as revealed by the second-order polynomial regression best fitted for all data points (solid line). The shaded area indicates the adjusting steps observed in animals receiving 6 µg and 8 µg of 6-OHDA.

also displayed similar degree of burstiness (see Materials and methods) as revealed by their NB (6 µg 6-OHDA: 91.92 ± 22.67 vs. 8 µg 6-OHDA: 101.67 ± 14.83) and PS values (6 µg 6-OHDA: 2.98 ± 0.61 vs. 8 µg 6-OHDA: 3.86 ± 0.73). These results indicate that despite the different degree of DA lesion induced with 6 and 8 µg 6-OHDA, both groups of animals showed similar electrophysiological changes in the SNpr (firing rate and firing pattern) (Fig. 3C and D). As illustrated in Fig. 3E, an enhanced proportion of SNpr LFO activity is correlated with the appearance of stepping performance deficits.

SNpr LFO units recorded from animals with severe DA denervation (> 95% DA depletion) are synchronized to the slow cortical rhythm

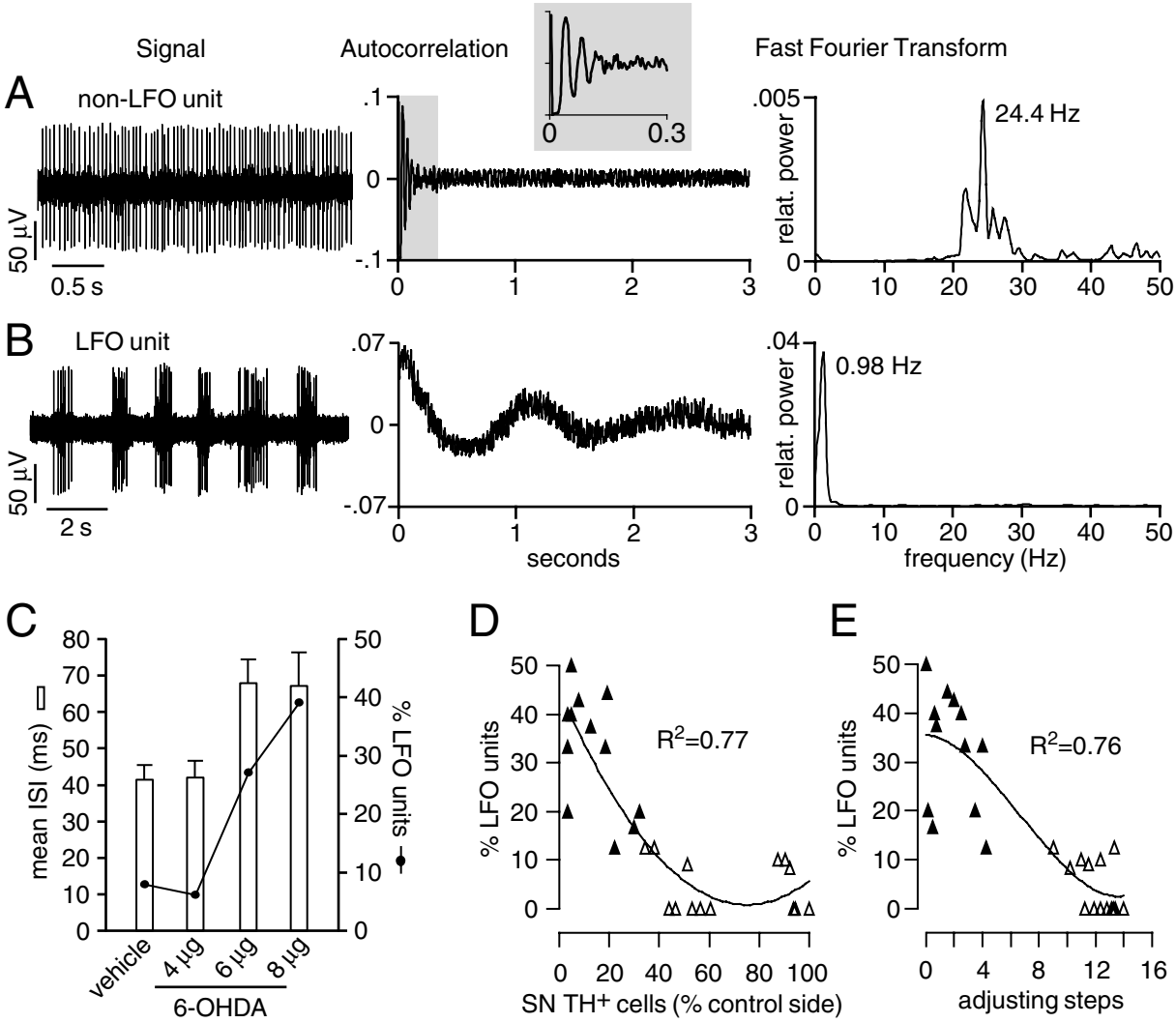

FIG. 3. Changes in SNpr neuronal activity induced by increasing doses of 6-OHDA. (A and B) Two representative examples of SNpr single-unit activity (left panel) recorded from animals that received 6 µg of 6-OHDA. As reported previously (Tseng *et al*., 2001a), neurons in the SNpr of urethane-anaesthetized animals could be classified into two main categories based on the autocorrelogram (middle panel) and the power spectra (right panel) of the interspike intervals: (A) neurons that display a tonic/regular firing pattern (non-LFO units); (B) those characterized by rhythmic burst of action potential (~0.9 Hz; LFO units). (C) Double *y*-axis plot summarizing the relationship between the mean ISI (left *y*-axis) and the proportion of LFO units (right *y*-axis) observed in the SNpr among groups of treatment. Note the similar mean ISI and percentage of LFO units between vehicle and 4 µg 6-OHDA neurons. By contrast, SNpr neuronal activity recorded from 6 and 8 µg 6-OHDA-treated animals exhibited similar increases in the proportion of LFO units and mean ISI. (D and E) Scatter plots illustrating the correlation between the proportion of SNpr LFO units in relation to the level of DA denervation (D) and the stepping test performance (E) observed in each animal. All 6 µg and 8 µg 6-OHDA-lesioned rats are indicated with closed triangles whereas the open triangles represent those from vehicle-treated and 4 µg 6-OHDA-lesioned groups. The solid lines indicate the second-order polynomial and sigmoidal (Boltzmann model) regression best fitted for all data points, respectively.

(Belluscio *et al*., 2003). To examine whether a similar degree of synchrony also exist among LFO units in 6 µg 6-OHDA-lesioned animals, ten LFO and 18 non-LFO units were recorded simultaneously with the frontal cortex ECoG in four rats. All comparisons were performed from similar cortical states, as revealed by the ECoG spectra showing dominant peak frequencies in the range of 0.5–1.5 Hz (ECoG from the LFO group: 0.78 ± 0.04 Hz; ECoG from the non-LFO group: 0.77 ± 0.04 Hz; Fig. 4, A1 and B1). Cross-spectra analysis showed that only neurons exhibiting an LFO firing pattern (spectral dominant frequency: 0.75 ± 0.05 Hz) oscillate synchronously with the cortical rhythm (Fig. 4, A1), with a coherence of 0.89 ± 0.03 at 0.72 ± 0.05 Hz (Fig. 4, A2). An equivalent level of synchrony also exists among six LFO/ECoG pairs (coherence: 0.84 ± 0.05, coincident oscillatory frequency: 0.76 ± 0.04 Hz) of three severely DA-lesioned animals (8 µg 6-OHDA). By contrast, no apparent coincident oscillatory activity was found among non-LFO/ECoG pairs (Fig. 4, B1 and B2). These results indicate that the firing pattern of SNpr LFO units from 6 µg 6-OHDA-lesioned animals is tightly linked to the cortical oscillatory activity, similar to that observed in the SNpr of severe DA-depleted brains (Belluscio *et al*., 2003).

Discussion

Early electrophysiological studies assessing oscillatory activity in the basal ganglia were obtained from animals with nearly complete mesencephalic DA neuron depletion. It remained to be determined whether less severe damage resembling a clinically relevant stage of Parkinson's disease could also give rise to substantial oscillatory activity. In the present study, chronic DA denervation was induced by

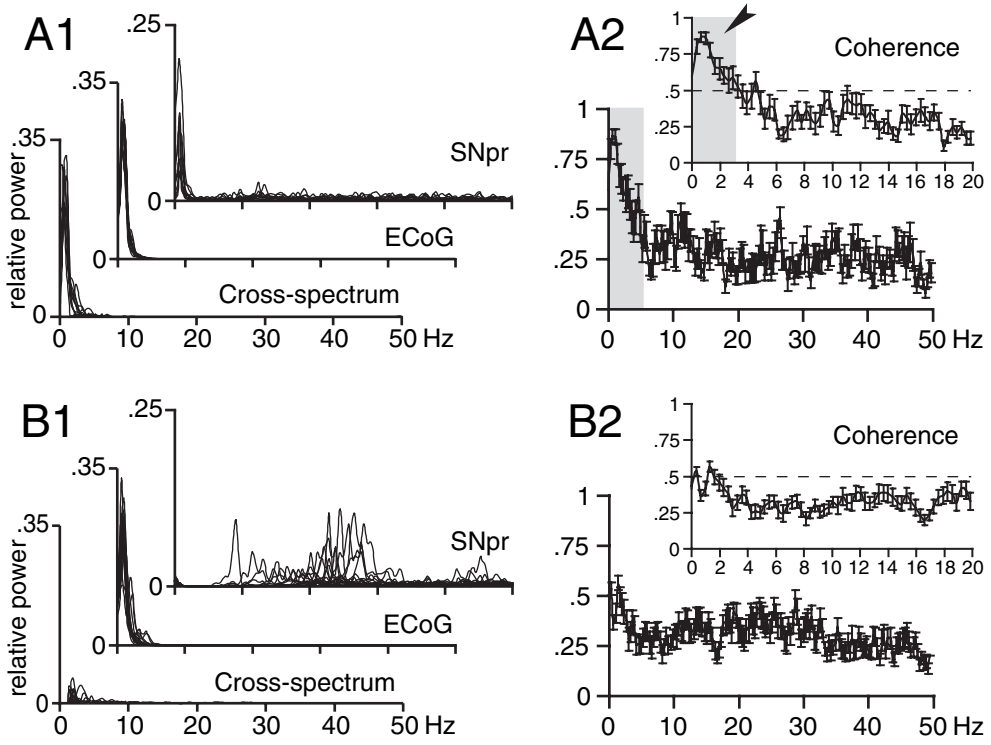

FIG. 4. Cortico-SNpr synchronous activity in 6 μg 6-OHDA-lesioned rats. Summary of the spectral density analysis obtained from 28 SNpr–ECoG pairs (ten LFO–ECoG pairs and 18 non-LFO–ECoG pairs) recorded in 6 μg 6-OHDA-lesioned animals. (A1 and B1) Power spectra plots showing the auto-spectra of each single unit (A1: LFO; B1: non-LFO) recorded simultaneously with the frontal ECoG, the auto-spectra of the ECoG, and their cross-spectra densities. (A2 and B2) Summary of the coherence estimation computed from the cross-spectra density of the signals. A significant coherence at the dominant frequency of the cross-spectra could be observed only among LFO–ECoG pairs (A2; arrowhead). No apparent coherent activity was observed in any non-LFO–ECoG pairs (B2).

injecting increasing doses of 6-OHDA into the medial forebrain bundle, which resulted in a dose-dependent decrease of TH^+ cells in both the SN and the VTA. Despite the extent of DA lesion (~60% DA neuron loss in the SN) induced with 4 μg of 6-OHDA, both the adjusting steps and the SNpr neuronal activity remained unchanged from those from vehicle-treated animals. A significant deficit in stepping test performance and an increased proportion of LFO units could be observed only following ~75% and ~55% reduction of SN and VTA DA neurons, respectively. These changes were not dramatically enhanced with 8 μg 6-OHDA, a dose that induced an extensive DA lesion (> 95%) in the SN and ~70% decrease of DA neurons in the VTA. These results suggest that the presence of LFO activity in the basal ganglia is not restricted to animals with almost complete DA neuron depletion. Instead, an increased proportion of LFO units emerges with less severe nigrostriatal damage and is correlated with the appearance of a motor deficit resembling akinesia.

It is well known that DA neuron loss in Parkinson's disease is progressive and that clinical manifestations do not appear until damage is extensive. In the last 25 years, diverse alternative models of 6-OHDA-induced DA lesion were developed to mimic this dynamic process in rodents. Depending on the aim of the experiment, these models have both advantages and limitations (Schwarting & Huston, 1996; Deumens *et al*., 2002; Schober, 2004). Here we attempted to investigate the electrophysiological consequences of different degrees of DA neuron depletion by lesioning the nigrostriatal pathway with different doses of 6-OHDA. The pattern of DA neuron loss induced by increasing doses of 6-OHDA injected into the medial forebrain bundle resembled closely that observed in the mesencephalon of parkinsonian patients (Fearnley & Lees, 1991; Gibb & Lees, 1991; Damier *et al*., 1999). With disease progression, the medial portion of the SN pars compacta and other medial mesencephalic DA cell groups, which are initially spared, become increasingly damaged (Fearnley & Lees, 1991; Damier *et al*., 1999). Similarly, injection of 4 μg 6-OHDA produced a DA lesion restricted to the middle–lateral region of the SN without affecting the VTA, whereas with 6 and 8 μg the degree of DA cell depletion increased in both the SN and the VTA in a dose-dependent manner. More importantly, higher doses of 6-OHDA produced a larger DA lesion in the SN than in the VTA, resembling the pattern of DA loss found in the parkinsonian brains (Fearnley & Lees, 1991; Damier *et al*., 1999).

We and others have reported that, in anaesthetized rats, chronic nigrostriatal lesion increases the proportion of basal ganglia neurons showing rhythmic firing rate modulations coupled to cortical oscillations (Magill *et al*., 2001; Tseng *et al*., 2001b; Belluscio *et al*., 2003). A DA-dependent coupling between electroencephalogram rhythms and local field potential activity at the subthalamic nucleus and globus pallidus was also reported in awake humans with Parkinson's disease (Williams *et al*., 2002) and behaving rats with 6-OHDA lesion (Sharott *et al*., 2005). Although the mechanisms that underlie this abnormal coupling are not fully understood, it appears that the firing pattern of an important population of neurons in the parkinsonian basal ganglia is strongly driven by oscillatory activity of the neocortex. It has been proposed that an enhancement of this cortically dependent oscillatory activity may disrupt information processing in the basal ganglia and the proper co-ordination and selection of afferent signals required to establish specific task-directed behaviours (Tseng *et al*., 2001b; Murer *et al*., 2002), and consequently that it may underlie the cardinal motor deficits of Parkinson's disease including tremor, akinesia and rigidity. This is supported by the fact that inactivation of the subthalamic nucleus alleviates parkinsonian signs

in humans (Bergman *et al*., 1990; Aziz *et al*., 1991; Wichmann *et al*., 1994; Limousin *et al*., 1995; Guridi *et al*., 1996) and reduces the proportion of LFO units in the basal ganglia of chronic DA lesion animals (Murer *et al*., 1997; Tseng *et al*., 2000, 2001a). Furthermore, pharmacological activation of DA receptors within the cortico-basal ganglia network also reduces the exaggerated cortically dependent oscillatory activity induced by chronic DA lesion (Murer *et al*., 1997; Tseng *et al*., 2000, 2004; Sharott *et al*., 2005). Altogether, these results indicate that an appropriate level of DA signal is required to maintain the proper temporal coupling and translation of afferent activity between the thalamo-cortical system and the basal ganglia nuclei. However, it is possible that the emergence of this abnormal cortico-basal ganglia synchrony occurs only in late-stage parkinsonism, especially as all previous studies were conducted in subjects with severe DA lesion (> 95% denervation). By examining the electrophysiological changes in the output nuclei of animals with different levels of DA neuronal depletion, we found that this was not the case. A significant increase of cortically driven bursting activity in the SNpr was observed in animals with ∼70% of mesencephalic DA denervation (∼75% in the SN and ∼55% in the VTA), but not in those with ∼60% of DA lesion in the SN with an intact VTA. Moreover, animals with > 95% and ∼70% loss of DA neurons exhibited similar stepping test deficits and showed comparable electrophysiological changes in firing rate, firing pattern and cortico-SNpr synchrony. A similar relationship was also observed between the level of DA lesion and the appearance of stepping deficits. The stepping test, as reported by Olsson *et al*. (1995), has repeatedly been used to evaluate akinesia (Kirik *et al*., 1998; Chang *et al*., 1999; reviewed by Deumens *et al*., 2002), the cardinal sign of Parkinson's disease. Previous reports stressed that partial lesions of the nigrostriatal system involving the sensorimotor striatal territory produce a significant deficit in the stepping test (Kirik *et al*., 1998; Chang *et al*., 1999), and that this deficit can be partially reversed by anti-parkinsonian therapies (Chang *et al*., 1999; Kirik *et al*., 2001; Fleming *et al*., 2005). Thus, the presence of this cortically dependent oscillatory firing pattern in the basal ganglia may represent an important pathophysiological feature of the parkinsonian state.

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Abbreviations

6-OHDA, 6-hydroxydopamine; DA, dopamine; ECoG, electrocorticogram; FET, Fisher exact probability test; FFT, fast Fourier transform; ISI, interspike interval; LFO, low-frequency oscillations; NB, number of bursts/3000 spikes; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PS, Poisson surprise; SNpr, substantia nigra pars reticulata; SN, substantia nigra; STN, subthalamic nucleus; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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