Dynamic Changes in the Cortex-Basal Ganglia Network After Dopamine Depletion in the Rat

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INTRODUCTION

The basal ganglia (BG) form a complex network that processes cortical information in the setting of motor control and learning (Graybiel et al. 1994). Classical models of Parkinson’s disease (PD) make use of the discharge rate in the BG structures and predict that dopamine (DA) depletion can exert opposite effects on the direct and indirect BG pathways, which convey cortical information from the striatum to the output BG structures. This prediction has been validated in the striatum

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METHODS

Animals

Four male Wistar rats (350–400 g, Drépat, Saint Doucald, France) were kept under standard housing conditions at constant temperature (22 ± 1°C), humidity (relative, 30%), and 12-hour light/dark cycles (daylight period 0800–2000 hours). Water was available ad libitum. Food intake was limited to 10–20 g/day to maintain constant animal weight. Animal care and surgery were consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the European community council directive of November 24 1986 (86/609/EEC) and was approved by the Comité Ethique de la Région Aquitaine.

Head stage

A custom head stage was designed for the purposes of this experiment to enable signals to be recorded simultaneously from the motor cortex anteroposterior (AP): +1.2 to +2.2 mm, mediolateral (ML): 1.5–2.5 mm, depth (D): 1.5–2.5 mm, the dorsal striatum (AP: 19 mm, 2 mg/kg, sc; Merial, Lyon, France) after surgery, and again 24 h later for pain relief. The animals were allowed to recover for 7 days.

Surgery

The rats were operated under xylazine (60 mg/kg, ip; Rompun, Bayer, Germany) and ketamine (100 mg/kg, ip; Virbac, Carros, France) anesthesia. Using a stereotaxic frame (Kopf), recording targets were located, above which holes were drilled in the skull. The head stage was lowered, and the holes were filled with petroleum jelly (Vaseline, Gifrer Barbezat, Decines, France). The head stage was attached to the animal’s skull with glue (Superbond, Sun Medical), dental cement (DentalonPlus, Heraeus Kulzer, Hanau, Germany), and stainless steel screws. Before the end of anesthesia, electrophysiological activities were recorded to make fine adjustments of the electrode bundle that contains the nigrostriatal dopaminergic fibers (−2.8 mm AP and +2 mm ML). The cannula length was adjusted so that once it had been inserted into the guide, its tip was positioned in the upper third of the medial forebrain bundle (D: −8.4 mm). The lesioning procedure is described below.

Data acquisition

Daily recordings ran for 1 h in a circular arena (40 cm diameter) during which physiological and behavioral activities were simultaneously recorded. Neural signals were preamplified 25 times (MiniHeadStage, AlphaOmega Engineering, Nazareth Illit, Israel) and amplified by a multichannel processor and digitized at a rate of 50 KHz (MCP, AlphaOmega Engineering). The raw signal was stored for further analysis at a lower rate of 12.5 KHz (AlphaMAP, AlphaOmega Engineering). In parallel, it was filtered (300 Hz to 3 KHz) for on-line spike discrimination using a template matching procedure (Multi Spike Discriminator, AlphaOmega Engineering). Discriminated spikes were stored synchronously with the raw signals. The animal’s movements were recorded simultaneously by a video tracking system (VTS, Plexon). Their position and the video recording were sampled at 30 Hz and stored separately from the neural data using video capture software (Cineplex, Plexon). Neural and behavioral signal recordings were triggered simultaneously, and the exact time of each position and video frame was sent to the AlphaMAP and stored in the neural data files for off-line synchronization.

At the end of the animals’ recovery period, after surgical implantation of the head stages, recordings were made under normal conditions for 2 wk. The nigrostriatal fibers were lesioned, and recordings were again made under the same conditions for a period of 3 wk.

6-OHDA unilateral lesion procedure

One hour before 6-OHDA injection, the animals were pretreated with intraperitoneal injections of desipramine (0.4% solution, 25 mg/kg, Sigma-Aldrich France, Lyon, France) and pargyline (0.1% solution, 5 mg/kg, Sigma-Aldrich France). Desipramine is used to protect the noradrenaline neurons from 6-OHDA, and pargyline potentiates the efficacy of 6-OHDA by inhibiting monoamine oxidase (Dunnett 1983). The animals were anesthetized with isoflurane gas (induction at 4% and then 2%) for the duration of the 6-OHDA injection. The injection cannula was inserted through the guide mounted on the head stage, and a Hamilton syringe was used to inject 6-OHDA into the medial forebrain bundle (8 μg in 1 μl of sterile water at a rate of 0.5 μl/min). After the injection had been completed, the syringe was left in place for 5 additional min to prevent the liquid from flowing back up the guide.

Histology

After the final recording, the rats were given a lethal dose of pentobarbital (Pentobarbital Sodique, CEVA, Libourne, France). Immediately after the injection, electrical microlesions (30 μA, 10 s) were induced by passing an anodal current through one electrode at each recording site. The brain was quickly removed and frozen in an isopentane bath at −80°C for histological analysis. Coronal brain sections (20 μm) were cut and those encompassing the motor cortex, striatum, and SNr were mounted on slides for electrode placement verifications. These slices were stained with cresyl violet for structural identification. The recording tracks and sites were established by observing the marks left by the cannulae and electrolesions (Fig. 1A).

The extent of the SNc lesion can be determined by measuring the relative amount of dopamine cell terminals in the striatum ipsilateral to the lesion compared with contralateral (Kunikowska and Jenner 2001). Dopamine transporters are present at the membrane of nigrostriatal dopamine cell terminals and give a indirect measure of the amount of SNc dopaminergic cells projecting to the striatum. Dopamine transporter binding procedure was performed as described previously (Bezard et al. 2001). After purification, [125I](E)-N-(3-iodo-prop-2-enyl)-2β-carboxymethyl-3β-(4’-methylphenyl) nortropane (PE2I) was obtained in a no-carrier-added form with a specific activity of 2,000 Ci/mmol and stored in ethanol at −20°C, a temperature at which it remains stable for 1 mo. Sections were incubated for 90 min at 25°C with 100 pM [125I]PE2I in pH 7.4 phosphate buffer (in mM: 10.14 NaH2PO4, 137 NaCl, 2.7 KCl, and 1.76 KH2PO4). After incubation, all sections were washed twice for 20 min in phosphate buffer at 4°C and dried at room temperature. They were exposed to radiation–sensitive film (Hyperfilm, Amersham, UK) in X-ray cassettes, for 7 days, for autoradiographic assessment of the radioactivity bound to regions of interest. The optical density was measured with an image analysis system (Densitag V. D2.00, Bioscom, Les Ulis, France) and averaged for each right and left striatum in each animal.

Signal processing

BEHAVIORAL ANALYSIS. Off-line discrimination of movement and rest episodes was carried out using Cineplex Markup software.
The choice of the motor parameter to measure to assess the impact of the lesion on the animals’ behavior was done in favor of locomotor activity. Locomotor activity has been shown recently to be decreased in rats bearing unilateral dopaminergic lesion (Steiner and Kitai 2001). Self-induced locomotor activity fits one important constraint of this study: to record physiological parameters in the unrestrained animal. For that reason we ruled out both the stepping test (Olsson et al. 1995) and drug-induced rotation tests.

Movement episodes consisted of periods during which the animal was moving (with the exception of grooming and rearing episodes). Basal locomotor activity was characterized by the average speed and SD (cm/s) during a typical rest episode for each rat. A threshold was defined as the upper confidence limit of the mean (see Statistical analyses). The animal was considered to be active when its instantaneous speed exceeded this threshold for >0.5 s. Rearing and grooming episodes were sorted manually using Cineplex Markup. The animal became accustomed to the recording chamber. For these reasons, rearing data are not discussed in this paper.

Analysis of the animals’ specific behavioral patterns during HVS episodes showed that they were awake and resting quietly while remaining responsive to tactile, auditory, and visual stimuli. Neither whisker twitching movements nor tremors were observed during HVS.

SPIKE DISCRIMINATION. The striatum contains several types of neurons, among which the medium spiny cells are the only output neurons. In this study, only putative medium spiny neurons (MSNs) discriminated according to shape and frequency criteria are described. Cells with a mean hyperpolarization duration >300 μs and a firing rate <2 spikes/s (n = 53) were classified as MSNs (Berke et al. 2004). Cells with a mean valley duration <300 μs and a firing rate >1 spike/s were classified as interneuron-like neurons. Cells not satisfying one of these two conditions were rejected. Very few interneurons (n = 3) were recorded during this study. As a consequence, results related to them are not presented here. All striatal neurons with waveform valleys <300 μs and mean discharge rates under 1 spike/s were rejected.

At the depth of 1.5–2.5 mm at which neurons have been recorded in this study, the cortex contains interneurons and pyramidal neurons (not necessarily sending axons in the pyramidal tract). Both types were discriminated using a time criterion (Bartho et al. 2004). Cells presenting a peak valley duration >500 μs were classified as putative pyramidal neurons (n = 62). Other cells were classified as putative interneurons (n = 2). Only pyramidal projection neurons were considered in this study. Corticostriatal neurons only represent a subset of...
projection neurons. The setup and recording procedure described here does not allow connectivity tests to be performed, such as antidromic stimulation or marker tracing, to detect whether neurons were actually sending axons to the striatum. It was assumed that part of the recorded cortical neurons were putative corticostriatal neurons. Moreover, simultaneous multiple single-unit recordings showed that projecting neurons (thus including corticostriatal) fire synchronously in a short time window during HVS (Kandel and Buzsáki 1997). Therefore, in our recordings, the overall firing time of cortical neurons is likely to be representative of the corticostriatal population itself.

The SNr contains a majority GABAergic neurons generating brief action potentials at a sustained firing rate and a few dopaminergic neurons, which when recorded extracellularly have been described as firing polyphasic long action potentials $>1.5$ ms at a slow rate $<8$ Hz (Hyland et al. 2002). Here the entire population of recorded neurons ($n = 76$) presented short biphasic extracellular action potentials (ranging from 0.81 to 1.14 ms) with a rather high firing rate $[18.4 \pm 1.9$ (SE) Hz]. Thus they entered the study considered as putative GABAergic projection neurons.

HVS DISCRIMINATION. HVS’s exhibit two main features, namely a spike and wave pattern and an oscillation frequency ranging between 5 and 13 Hz (Fig. 1B). Both criteria were used to detect the beginning and end of these episodes. For frequency criteria, a threshold on the instantaneous power spectral density (PSD) of local field potentials (LFPs) was used. LFPs were first filtered (1–200 Hz) using a second-order band-pass Butterworth filter and down-sampled (500 Hz). For each structure, the PSD was computed every 100 ms using an overlapping sliding window with a length of 250 points (0.5 s). The instantaneous average PSD in the 5- to 13-Hz range was computed for each time step. Its value was deemed to have increased significantly when it passed the confidence limit (see Statistical analysis). Overthreshold time steps determined preliminary epochs. A pattern criterion was assessed manually. Preliminary epochs, within which the LFPs showed a typical spike and wave pattern, were rejected. Previous studies showed that HVS onset and offset could occasionally be different in cortex and striatum (Berke et al. 2004). This lag can be attributed to the fact that onset and offset may vary across cortical locations (Polack et al. 2007; Shaw 2004). Since the cortical recording electrode was placed at a given location, the HVS could have been transmitted first from another cortical location to the striatum, thereby introducing an apparent onset delay. The same phenomenon could account for a delay in observed offsets. The analysis time frame was restricted to one oscillation cycle, where the cortex, striatum, and SNr were all oscillating. To remove the lag-related bias, only those time intervals having overlapping candidate epochs were taken into account, using Neuroexplorer software (Nex Technologies, Littleton, MA).

HVS oscillation trough and peak markers were discriminated within HVS epochs using temporal and voltage criteria with a C+ homemade routine running under MathLab (The Mathworks, Natwick, MA). Cortical electrodes were placed around the border of layer V and VI ($\sim 1.5$–$2.5$ mm below the surface) to record projection neurons. At this level, the earliest HVS spike component is negative (Kandel and Buzsáki 1997). For this reason, cortical LFP markers were positioned at the minimum voltage in the troughs. Because striatum and SNr spike components are positive, striatal and nigral markers were positioned at the maximum voltage in the peaks.

Spectral characterization of LFP

The PSDs of LFPs were computed using fast Fourier transform (FFT) analysis and Welch method as spectral estimator with a sliding windows of 1,250 samples (2.5 s), over the frequency range from 0 to 500 Hz (0.39-Hz resolution). Histograms were smoothed using a three-point Gaussian process.

Coherence was computed using the expression

$$\text{Coherence}_{ij} = \frac{P_{\text{xx}} \times P_{\text{yy}}}{P_{\text{xx}} \times P_{\text{yy}}}$$

where $P$ is the average of the squares of the LFP spectra $i$ and $j$. These
spectra were computed using the same method as described above (unless otherwise stated). Comparisons between coherence histograms computed during rest, movement, and HVS episodes were made using a one-way ANOVA (with $P = 0.05$).

**Characterization of spike trains**

Cross-correlograms were computed using a bin size of 2 ms with 50 randomly chosen action potentials to avoid analytic bias related to
potential differences in sample size, especially when comparing before and after the lesion. Cross-correlogram PSDs were computed by FFT, using sliding windows of 256 samples (1.28 s) over the frequency range 0–200 Hz, yielding a resolution of 0.8 Hz. Histograms were smoothed using a three-point gaussian process. The confidence limit (CL) of the PSD histograms was computed over the 3- to 50-Hz range (see Statistical analysis), and autocorrelograms and cross-correlograms were considered oscillatory if any PSD value in the spectral window from 5 to 13 Hz passed the CL (with $P < 0.01$).

**Time lag distributions**

Peri-event histograms of the discharge activity of single neurons and of the next cortical LFP markers were constructed, using the HVS cortical marker as a trigger ($t_c$), over a time frame of 100 ms before and after $t_c$. For the purposes of graphical representation, a 2-ms bin size and a three-point gaussian smoothing algorithm were used. For this analysis only, triggers and spike trains that occurred $>50$ times in the HVS episodes were considered. A peak was considered significant if it exceeded the upper CL (see Statistical analysis), and a trough was considered significant if its value was below the lower CL. The presence of a significant peak and/or trough was used as a criterion in defining a neuron to be “HVS driven.” For the HVS driven neurons, peak and trough times were averaged to construct population time lag distributions in the time range of 50 ms before and 140 ms after cortical LFP marker.

**Phase relationship**

To determine the phase relationship between neurons and cortical LFPs following the method used by Klausberger et al. (2003), we used a Matlab algorithm (see HVS discrimination section above) to detect the troughs of HVS. Each spike was assigned to a phase between the troughs of HVS. Each spike was assigned to a phase between the troughs $n$ and $n + 1$ given that the peak of LFPs was arbitrarily assigned the angle value $0^\circ$. The circular space was divided into 100 bins of equal size giving a resolution of 3.6°. The significance of the phase relationship was analyzed using the Rayleigh test for directional data (Fisher 1993). This tests the hypothesis that spike phases are uniformly distributed along the circular space ($0–360^\circ$). For each spike train, this test was carried on 50 randomly chosen action potentials to test samples of the same size and avoid analytic bias related to differences in sample size. The preferred phase (i.e., phase represented by the highest number of spikes) was collected to construct and compare the phase distributions for neurons of each structure before and after dopamine depletion. This phase analysis of neuronal spike trains showed that the neurons that presented a significant bias toward a preferred phase of cortical LFPs were also the ones that were defined as HVS driven by the time lag analysis (previous section). This shows that firing rate of neurons selected for this study had no influence on their oscillatory behavior.

**Statistical analysis**

Statistical analyses were performed with Sigma Stat software (Version 2.03, SPSS, Chicago, IL) and GraphPad Prism (version 4.00, GraphPad Software, San Diego CA). A probability level of 5% ($P < 0.05$) was considered significant. Variables are presented in the following form: means ± SE. CLs were computed as CL = mean ± 3 SD. Comparisons between mean locomotor activity were made using a two-way ANOVA (factor 1: selected rat; factor 2: control/lesion) to assess the influence of the extent of the lesion in each rat. HVS duration and number and LFP oscillation frequencies were assessed using a paired t-test (normal and dopamine-depleted animals). Coherence was analyzed using a two-way ANOVA (factor 1: selected rat; factor 2: pair of structures) followed by post hoc multiple comparisons using the Holm-Sidak method and a paired t-test to assess the influence of the lesion on the level of coherence. Oscillatory AC and CC percentages were analyzed using $\chi^2$ tests (normal and dopamine depleted). Firing rates during and outside HVS episodes were compared using a Mann-Whitney rank sum test. AC mean oscillation frequencies were compared using a t-test (normal and dopamine depleted). Firing rates presented nonnormal distributions and were therefore analyzed using a nonparametric test: a Mann-Whitney rank sum (normal and dopamine depleted) and a one-way ANOVA on ranks (total, oscillatory, and nonoscillatory), followed by post hoc multiple comparisons using Dunn’s method. Time lags between neuron firing peaks and LFP peaks presented non-normal distributions and unequal variances. They were therefore analyzed using a Mann-Whitney rank sum test (normal and dopamine depleted).

The comparison of phase distributions in the circular space for control and lesion condition was done using the Watson U2 test (Fisher 1993).

The locomotor activity is expressed as a percentage of the time spent moving under control conditions. ACs and CCs are presented as a percentage of the total AC and CC, respectively.

**RESULTS**

**Extent of striatonigral lesion and behavioral impairment**

In the striatum ipsilateral to the lesion, the optical density for the striatum decreased by an average of 78.2 ± 6.9% (range, 68.5–90.9) compared with the contralateral hemisphere (see example on Fig. 1C). Under control conditions, the animals spent 20.6 ± 0.8% of their time moving (range: 13.7–30.1%). Three weeks after the lesion was processed, the percentage of time spent moving decreased significantly, by an average of 50%, to reach 9.5 ± 0.5%. The lesion was the significant factor influencing the drop in locomotor activity explaining 73% of the variance, whereas the animal only accounted for 14% [2-way ANOVA, $F(1,19) = 123.78$, $P < 0.05$ and $F(19,19) = 1.41$, $P = 0.23$, respectively, with no interaction]. Therefore the lesion procedure induced a marked homogeneous behavioral impairment in all the animals.

**Effects of dopaminergic depletion on HVS features**

Under normal conditions, the mean duration of a HVS was 1.1 ± 0.3 s (Fig. 2A), and the mean number of episodes was 30.7 ± 6.3 per hour and 52.0 ± 8.3 per hour of rest (Fig. 2B). The HVS mean frequency was 8.8 ± 0.4 Hz, with a frequency range extending from 5 to 13 Hz (Fig. 2C). During HVS, the coherence in the range 5–13 Hz between the different pairs of LFPs was 0.47 ± 0.03 in cortex-striatum pairs, 0.30 ± 0.03 in striatum-SNr pairs, and 0.26 ± 0.04 in cortex-SNr pairs (Figs. 3 and 4), such that cortex-striatum coherence was significantly higher than in the case of striatum-SNr and cortex-SNr. The latter was not significantly different [2-way ANOVA, selected-rat factor: $F(3,12) = 1.54$, $P = 0.56$ and pair-of-structures factor: $F(2,12) = 5.42$, $P < 0.05$; Holm-Sidak post hoc comparison method: $P < 0.05$, $P < 0.05$ and $P = 0.57$ for cortex-striatum, striatum-SNr, and cortex-SNr pairs respectively].

Under parkinsonian conditions (as shown for example in Fig. 3, B and C), the mean duration of HVS episodes increased significantly (Fig. 2A; 2.6 ± 0.2 s; paired t-test, $P < 0.05$) as did the number of episodes per hour and per hour of rest (Fig. 2B; respectively, 72.2 ± 4.7 and 86.7 ± 3.5, paired t-test: $P < 0.05$ and $P < 0.05$). The HVS mean frequency was not significantly modified (Fig. 2C; 9.6 ± 0.5 Hz; range, 5–14 Hz).
Dopaminergic depletion enhances neuronal rhythmic firing

Under normal conditions, 25 neurons were recorded in the motor cortex, 17 MSNs in the striatum, and 31 neurons in the SNr. Firing rates during HVS were 4.1 ± 1.3 spikes/s in the cortex, 0.8 ± 0.2 in the striatum, and 18.4 ± 1.9 in the SNr (Fig. 5). On average, no significant changes were observed between HVS episodes and other epochs of our recordings (paired t-test: $P = 0.976$, $P = 0.759$, and $P = 0.489$, respectively). During HVS under normal conditions, the average oscillation frequency of the neuron autocorrelograms (Fig. 2C) was not significantly different from that observed in the LFP ($t$-test: $9.5 ± 0.3$ Hz, with a range extending from 6.1 to 11.4, $P = 0.209$). The proportion of oscillatory autocorrelograms was 45% in the cortex, 30% in the striatum, and 55% in the SNr (Fig. 6, A–C). The proportion of oscillatory intrastucture cross-correlograms was 62% in the cortex, 35% in the striatum, and 60% in the SNr (Fig. 6, A, B, and D). Interstructure cross-correlogram analysis showed that 22% of cortex-striatum pairs of neurons, 51% of striatum-SNr pairs, and 53% of cortex-SNr pairs were oscillatory. As expected, auto- and cross-correlogram percentages were significantly higher during HVS than during the other recording periods, in which the number of significant synchronizations and oscillations hardly reached 5% ($\chi^2$ test: $P < 0.05$, data not shown).

Under parkinsonian conditions, 37 neurons were recorded in the motor cortex, 36 MSNs in the striatum, and 46 neurons in the SNr. The firing rate (Fig. 5) in the cortex and striatum did not change ($2.5 ± 0.4$ spikes/s, Mann-Whitney rank sum test: $P = 0.154$; $0.8 ± 0.1$ spike/s, Mann-Whitney rank sum test: $P = 0.851$, respectively), whereas it increased significantly in the SNr ($25.4 ± 1.9$, Mann-Whitney rank sum test: $P < 0.05$). On average, no significant changes in firing rate were observed between the HVS episodes and other epochs of our recordings (Mann-Whitney rank sum test: $P = 0.705$, $P = 0.850$, and $P = 0.930$, respectively). The percentage of oscillatory autocorrelograms increased significantly to reach 76% in the cortex, 58% in the striatum, and 89% in the SNr (Fig. 6, A–C). The average oscillation frequency observed in these autocorrelograms (Fig. 2C) did not significantly differ from that observed under normal conditions ($10.3 ± 0.3$ Hz with a range extending from 7.4 to 13.1, $t$-test: $P = 0.129$). The percentage of oscillatory intrastucture cross-correlograms increased significantly, reaching 86% in the cortex, 78% in the striatum, and 81% in the SNr ($\chi^2$ test: $P < 0.05$, $P < 0.05$, and $P < 0.05$, respectively).
A. Oscillatory cross-correlograms (CC) of pairs of simultaneously recorded neurons. For a majority of neurons, the period of discharge oscillation was altered by DA depletion for each pair of structures (Fig. 6D). The percentages of oscillatory cross-correlograms (CC) of pairs of neurons recorded in the cortex, striatum, and SNr. For C and D, *significant difference between values under control and lesioned conditions, as assessed with a t-test. DA depletion significantly increased the percentage of oscillatory neurons and that of oscillatory synchronization between neurons.

Alteration of the temporal organization of neuron discharges in DA-depleted rats

As shown in the example of Fig. 7, A–C, examination of the peri-event histograms showed that a majority of neurons in each structure was driven by the cortical rhythm during HVS, under both control conditions and after lesion of the nigrostriatal pathway (Table 1). The peri-event histograms allowed us to detect rhythmicity in the MSNs, which, as a consequence of their sparse firing activity, was not shown by the autocorrelation function.

Under control conditions, when timed with respect to the cortical HVS marker, cortical firing peaked at $-5.8 \pm 4.2$ ms, striatal activity peaked at $3.2 \pm 3.9$ ms, the minimum of SNr activity occurred at $15.6 \pm 3.9$ ms, and SNr activity peaked at $40.5 \pm 4.8$ ms (Fig. 7D). In the cortex and striatum, these

![Image of oscillatory cross-correlograms](http://jn.physiology.org/)

**TABLE 1.**

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<th>Cortex</th>
<th>Striatum</th>
<th>SNr</th>
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<tr>
<td>Control</td>
<td>16/25</td>
<td>10/17</td>
<td>17/30</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>27/37</td>
<td>22/36</td>
<td>40/46</td>
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*Significant peak.*
results are consistent with other studies of freely moving animals (Berke et al. 2004; Kandel and Buzsáki 1997). To the best of our knowledge, equivalent data have not been reported for SNr neurons. After DA depletion, the pattern of activation was significantly altered only in SNr: the minimum and maximum of SNr activity occurred respectively earlier at $-24.3 \pm 4.4$ and $10.2 \pm 1.07$ ms, respectively, with respect to the cortical HVS marker. Moreover, the percentage of SNr neurons exhibiting troughs significantly fell from 58% before the lesion to 34% afterward ($\chi^2$ test, $P < 0.05$). In contrast, the percentage of SNr neurons exhibiting peaks significantly increased from 58 to 87% ($\chi^2$ test, $P < 0.05$). No significant shifts were observed in the case of cortical and striatal neurons (Mann-Whitney rank sum test: $P = 0.972$ and $P = 0.921$, respectively). The period of the oscillations, computed with the intervals between the cortical HVS trough markers (Fig. 8, A and D), also did not change before and after the lesion ($t$-test, $P = 0.740$). This indicates that the firing peak shift observed in the SNr was not caused by a decrease in oscillation period.

Alteration of the phase of neuron discharges in DA-depleted rats

The phase analysis showed that a majority of neurons displayed a bias toward a preferred phase of the cortical LFPs (Rayleigh test). Moreover, the population of oscillatory neurons matched exactly the population of ‘HVS driven’ neurons.

The SNr neuron phases are altered toward smaller values after the lesion with a significant shift from $124 \pm 15$ to $34 \pm 5^\circ$ (Watson U2 test, $P < 0.05$). On the contrary, the phases of cortical (control: $-14 \pm 11^\circ$; lesion: $-9 \pm 5^\circ$) and striatal neurons (control: $8 \pm 12^\circ$; lesion: $18 \pm 9^\circ$) action potentials remained stable (Fig. 8; Watson U2 test, $P > 0.05$).

Discussion

In this study, we examined HVS to compare connectivity in the cortex-BG network before and after dopaminergic depletion. The main finding using this original approach is the demonstration that the temporal distribution of discharge activity in the network is altered after dopaminergic lesion: nigral neurons responded significantly earlier to cortical activation. Moreover, DA depletion induced an overall increase in synchronization in the network during HVS, and this effect was particularly pronounced in the case of striatal output neurons.

DA depletion and locomotor impairment

In our study, the average level of striatal dopaminergic terminal loss was >68%. We observed both an increase in oscillatory activity during HVS and a decrease in locomotor activity. This is consistent with a recent study showing that akinesia and changes in the dynamic properties of BG neurons appear when striatum presents a decrease of >70% of dopaminergic terminals (Tseng et al. 2005). The decrease in motor activity (55%), observed 20 days after the lesion was intro-
enhancement of oscillations in a subpopulation of MSNs. On the whole, our observations related to HVS are in agreement with numerous publications, showing that synchronous oscillations are enhanced in the presence of parkinsonian conditions (Belluscio et al. 2003; Bergman et al. 1994; Meissner et al. 2005; Sharott et al. 2005). An alternate hypothesis has been evoked to explain increased synchronized oscillations in parkinsonian conditions. A popular one is the reverberating STN-GPe loop (Bevan et al. 2002); however, in our case, the shortening of the latency between cortical and SNr responses and of the phase of the SNr neuronal responses rendered this hypothesis very unlikely as far as HVSs are concerned. We nevertheless do not rule out that these mechanisms play a role in the stabilization and/or the generation of other type of oscillations.

**DA depletion increases the number and duration of HVSs**

After DA depletion, HVS spindles are significantly longer and more numerous than under normal conditions, and the coherence between each pair of structures is increased. Our observations are consistent with previous studies, showing that alteration of the BG leads to modification in the occurrence of HVS, although they are not involved in their generation (Deransart et al. 1998). Indeed, the number and duration of HVS have been shown to be diminished by the injection of dopaminergic agonists inside the striatum (Deransart et al. 2000). The increase in number and duration of HVS observed after DA depletion is thus in line with previous observations by Deransart et al. (2000) and could be caused by the enhancement of oscillatory synchronization in the cortex-BG network, which we have shown is particularly pronounced in the striatum.

**DA depletion qualitatively changes the top-down transmission of information**

Studies carried out in rats and monkeys have shown that frontal or prefrontal cortical stimulation can elicit complex responses in the BG output structures, the SNr, or the globus pallidus pars interna (Kolomiets et al. 2003; Maurice et al. 1999; Nambu et al. 2000). These authors have reported triphasic responses in the majority of SNr neurons characterized by an early excitation, followed by an inhibition and a late excitation. Each component of this response has been attributed to the activation of one of the three BG pathways with, in chronological order, the trans-subthalamic hyperdirect pathway (excitatory, between ~7 and ~16 ms), the trans-striatal direct pathway (inhibitory, between ~10 and ~30 ms), and the indirect pathways (excitatory, between ~25 and ~50 ms). With our approach, under normal conditions, the trough in SNr activity follows the cortical peak by 15.6 ms, and the SNr peak occurs 40.5 ms after the cortical peak. On the basis of these values, we have previously suggested that during HVS, SNr activity is driven by inputs from the direct and indirect pathways, but not by the hyperdirect pathway (Dejean et al. 2007).

After dopaminergic depletion, we observed two major phenomena: 1) the SNr trough no longer seemed to be driven by the cortical rhythm, because this feature was no longer observable in more than third of the neurons and it also occurred 23.4 ms earlier than the cortical HVS; and 2) the SNr firing peak occurred significantly earlier, i.e., its delay after the cortical

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**FIG. 8.** Phase relationship between cortical, striatal, and SNr spikes and cortical LFPs. **A:** the polar plots summarize the distributions of the preferred phase of all oscillatory neurons in cortex (top), striatum (middle), and SNr (bottom) with regard to cortical LFPs (angle 0° corresponding to LFPs peak). Neurons recorded before the lesion are represented in gray and those recorded after the lesion are plotted in black. **B:** examples of firing as a function of phase for single neurons recorded before (gray) and after (after) the lesion. The firing rate scale indicates the average firing rate in hertz per bin (3.6°). Ticks and corresponding angle value on the outer cycle show the preferred phase of the neurons.

**DA depletion increases cortex-BG synchronization during HVS**

We observed that neuronal oscillatory and synchronous activity is increased in all structures and most notably in the striatum. This increase is consistent with previous studies of the striatum (Tseng et al. 2001) and SNr (Belluscio et al. 2003) of anesthetized rats. In agreement with studies in the parkinsonian monkey (Goldberg et al. 2002; Nini et al. 1995), we also observed an increase in intracortical and intranigral neuronal synchronization. We have shown that the level of interstructure oscillatory synchronization in the BG increases, whichever pair of structures is considered, both in the HVS (5–13 Hz) and the beta band (15–30 Hz). This increase is particularly pronounced in pairs involving striatal neurons and is likely caused by the...
HVS marker decreased from 40.5 to 10.2 ms. The SNr peak thus occurs inside the time window of the electrically evoked responses mediated by the hyperdirect pathway (Kolomiets et al. 2003; Maurice et al. 1999; Nambu et al. 2000). However, the duration (40 ms) of the excitatory response observed after DA depletion suggests that this form of excitation is not only mediated by the hyperdirect pathway but is also merged with a late excitatory component likely caused by the activation of the indirect pathway. In conclusion, these alterations may be caused by a decrease in efficiency of the direct pathway, associated with an increase in that of the hyperdirect and indirect pathways. Numerous lines of evidence in the literature support this conclusion. On the one hand, a weakening of the direct pathway is in line with classical models of the pathophysiology of PD (Albin et al. 1989; DeLong 1990). This view has returned to the forefront of BG research with very recent studies using cortical stimulation in anesthetized animal. Indeed Mallet et al. (2006) have shown that DA depletion strongly depresses the response of striatonigral-direct-pathway neurons to cortical input. Moreover, two other groups showed a decrease of direct pathway influence on SNr neurons in both the unilateral 6-OHDA model used here (Belluscio et al. 2007) and an acute model of PD (Degos et al. 2005). On the other hand, it has been shown that DA depletion facilitates the transmission of cortical oscillations to the STN and the globus pallidus in the rat. It has been further suggested that both hyperdirect and indirect pathways were strengthened (Magill et al. 2001; Walters et al. 2007). Importantly, strengthening of the indirect pathway is also in agreement with classical models and has recently been shown by electrophysiological and anatomical studies in the striatum (Day et al. 2006; Mallet et al. 2006). These studies support the presence of an imbalance between striatonigral and striatopallidal neurons as stressed in the classic models. In this study, we observed that rhythmically driven MSNs showed significantly higher firing rates than nondriven units under parkinsonian conditions. Rhythmically driven and nondriven neurons could therefore correspond to striatopallidal and striatonigral MSNs, respectively.

We propose that the loss of striatal inhibition unmasks the earliest excitatory components, caused by the indirect pathway. In this study, we also observed a possible overexpression of the hyperdirect pathway in the SNr. The removal of direct inhibitory influence may leave a time window open for hyperdirect inputs, which were probably overshadowed by striatal inhibition under control conditions (Dejean et al. 2007).

Striatal gating of oscillatory signals

As in previous studies, we showed that, despite the strong coherence observed between cortical and striatal LFPs (Mahon et al. 2001; Slaght et al. 2004; Stern et al. 1997), oscillations in the spiking activity of MSNs are weak (for a detailed discussion of this point see Berke et al. 2004; Dejean et al. 2007). This lack of oscillatory output can explain the discrepancy between the observed level of coherence between cortex and striatum and that between either striatum and SNr or cortex and SNr. However, after 6-OHDA lesion, these differences in coherence level disappeared. Moreover, the striatal MSNs show the greatest increase in oscillatory and synchronized firing behavior. Our results suggest that DA depletion facilitates the transmission of oscillations through the striatum. This is consistent with recent theories of BG in which the striatum acts as a gate for information flowing toward downstream structures (Murer et al. 2002; O’Donnell 2003).

Conclusion

Among various symptoms, patients with Parkinson’s disease present a strong impairment in the initiation of movement and a marked slowing of reaction times (Agid 1991). We have recently shown that the latter is correlated with a paradoxical shortening of the response latency of BG output neurons: in the MPTP monkey the neurons of the globus pallidus pars interna respond earlier to a “go-movement” stimulus (Leblois et al. 2006). These results provide an explanation for this finding by showing that it may rely on an alteration of the computation of information from the concurrent BG pathways.

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References

Degos B, Deniau JM, Thiiery AM, Głowinski J, Pезard I, Maurice N. Neuroleptic-induced catalepsy: electrophysiological mechanisms of func-


