Behavioral and Anatomical Effects of Long-Term L-Dihydroxyphenylalanine (L-DOPA) Administration in Rats with Unilateral Lesions of the Nigrostriatal System

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This study investigated behavioral and anatomical changes induced by long periods of L-DOPA treatment in the unilateral rat model of Parkinson’s disease. After daily injections of L-DOPA (50 mg/kg, ip) given for 1, 4, 8, or 16 weeks, behavioral sensitization, expressed by contralateral turning and changes in its pattern, increased within the first week of treatment and remained unchanged thereafter. Dyskinetic movements, affecting the trunk and limbs of all treated rats, also developed within the first week of treatment and increased further during the 16 weeks of L-DOPA treatment. L-DOPA responsiveness was also accompanied by changes at the neuronal level, as shown by changes in the expression of c-fos in the dopamine-depleted striatum. Following 1 week of L-DOPA treatment there was a marked decrease in striatal c-fos expression, compared to single injections, especially evident in the medial and ventral regions and to a lesser extent in the dorsolateral regions of the striatum. This specific regional expression of c-fos was maintained throughout the 16 weeks of L-DOPA treatment. Overall, our results show that behavioral sensitization to L-DOPA starts relatively early during the treatment and include not only an increase in contralateral turning rate but also an increase in dyskinetic movements. Persisting c-fos expression in the dorsolateral striatum might be implicated in the development of dyskinesias when L-DOPA treatment is extended for periods longer than 1 week.

Key Words: striatum; 6-OHDA lesions; L-DOPA; turning; dyskinesias; c-fos.

INTRODUCTION

The dopamine (DA) precursor L-DOPA is still the most common and effective therapeutic approach used to reverse the symptoms of Parkinson’s disease (PD). Nevertheless, after several years of treatment PD patients develop unwanted complications such as rapid and often unpredictable changes in L-DOPA responsiveness, and a variety of severe dyskinetic movements characterized by disorganized or excessive involuntary, stereotypic, and even ballistic movements. The relationship between L-DOPA treatment and these side effects is still unclear and the use of L-DOPA in PD has been debated (1, 6, 11, 43).

Several factors may play a role in the development of L-DOPA side effects. Some studies have suggested a direct relationship between the duration of the treatment and the progression of L-DOPA-induced dyskinesias (4, 26, 35, 36). This effect may be related to increased DA receptor sensitivity occurring after extensive DA depletion (8, 19, 26, 54) and to decreased L-DOPA efficacy in normalizing functional changes due to progressive DA denervation (12, 24, 33). Nevertheless, besides a few early studies in which L-DOPA was orally administered for several months to normal rats (41, 48), there have not been long term studies analyzing behavioral and neuronal changes that occur following L-DOPA treatment in the rat model of PD.

Most of the experimental work that has investigated the dynamics of L-DOPA effects have used the turning response in the rat after unilateral nigrostriatal lesions, i.e., the unilateral PD rat model (42, 50, 53). Repeated L-DOPA administration to DA-depleted rats has been found to induce behavioral sensitization expressed by increased contralateral turning (3, 9). In addition to turning, DA-depleted rats treated with L-DOPA display a complex repertoire of stereotypic and dyskinetic movements (10, 34, 50). This suggests that in order to understand the effects of long-term L-DOPA treatment the analysis of this animal model should include a detailed assessment of all behavioral changes that develop during the treatment, not only turning.

It has been shown that enhanced behavioral response to repeated DA-replacement therapy seen in
the rat model of PD has pharmacological and behavioral characteristics similar to L-DOPA-induced dyskinesias in man and in MPTP-lesioned monkeys (9, 19, 22, 33, 44). Therefore, using a unilateral rat model of PD, we investigated the development of dyskinetic movements during 4 months of L-DOPA treatment, along with changes in turning behavior. In this study we used animals with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal system. The L-DOPA-induced behavior was monitored across the whole length of the treatment, in the home cage and in flat circular arenas to allow video recording and quantification of the behavior after the very first and last L-DOPA injection.

Recent results using animal models of PD linked changes at the gene level to behavioral changes following L-DOPA treatment (3, 6, 9, 19). In addition to inducing behavioral responses, L-DOPA stimulates the expression of the immediate-early gene c-fos, which is considered a marker for neuronal activity in the striatum of PD rats treated with L-DOPA (15, 45, 47). Whereas L-DOPA given acutely to rats with a unilateral DA depletion is known to induce extensive expression of c-fos (45), repeated L-DOPA administration (10–15 days) has been found to decrease c-fos expression in the lesioned striatum. The question we are asking is whether c-fos is completely abolished by prolonged treatment with L-DOPA, which may be related to loss of L-DOPA efficacy. In addition, we want to compare the occurrence and the striatal distribution of c-fos to the changes in L-DOPA-induced behavior throughout 4 months of L-DOPA treatment.

MATERIALS AND METHODS

Unilateral 6-OHDA Lesions

Male Wistar rats (250–300 g, WIST Zur, Institute of Toxicology, Schwerzenbach, CH) were housed under a 12-h reversed light/dark cycle with free access to food and water. All rats underwent unilateral injections of 6-OHDA in the medial forebrain bundle (MFB). Animal care procedures and experimental protocols were approved by the Ethics Committee of the Veterinary Office of the Canton of Zurich, Switzerland, and were in accordance with NIH guidelines.

Thirty minutes before surgery, rats (n = 84) were given pargyline (40 mg/kg, ip) to prevent the metabolism of 6-OHDA. They were then anesthetized with pentobarbital (Nembutal 50 mg/kg, ip) and placed in a stereotaxic frame. The skull was exposed and a hole was drilled to deliver 6-OHDA unilaterally at the level of the right MFB: 4 mm posterior to Bregma, 2 mm to the right of the midline, and 8 mm ventral to the surface of the cortex. 6-OHDA (8 μg in 2 μl) was injected into the MFB through a 28-gauge stainless-steel cannula connected by a Teflon tube to a 10-μl Hamilton syringe. The injection speed was 1 μl/min, and the cannula was left in place for an additional 3–5 min postinjection. After the lesion, animals were allowed to recover for 2 weeks.

L-DOPA Treatment

To avoid drug administration while testing for the presence of >90% DA depletion (see priming effect, Morelli and Di Chiara (31)), 2 weeks following the lesion rats were tested for contralateral tactile neglect and spontaneous ipsilateral postural asymmetry, with respect to the lesion side. The majority of the animals (n = 78) showed no response to contralateral tactile stimuli and showed the presence of ipsilateral postural asymmetry after stress stimuli or while climbing a vertical grid, and were included in the study. Six rats were then excluded.

Animals with unilateral lesions were treated either chronically or acutely with L-DOPA methyl ester. In the chronic study (n = 48), 4 L-DOPA-treated groups (n = 4 × 10) were given daily injections of L-DOPA plus benserazide (50 mg/kg, ip and 25 mg/kg, respectively) and 4 control groups (n = 4 × 2) were treated daily with saline for 1, 4, 8, or 16 weeks. The treatment started 2 weeks after the lesion and terminated 3, 6, 10, or 18 weeks after the lesion (see Table 1). In the acute study (n = 30), 5 L-DOPA-treated groups (n = 5 × 4) were given a single injection of L-DOPA plus benserazide, and 5 control groups (n = 5 × 2) were given a single injection of saline at 2, 3, 6, 10, or 18 weeks postlesion (see Table 1). All drugs used were purchased from Sigma, Buchs, Switzerland.

Behavioral Testing

In the chronic study, animals were treated with L-DOPA once a day and their behavior was observed in their home cage. Nonresponding episodes ("no" re-

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<td><strong>L-DOPA Treatment Schedule for the Acute and Chronic Groups</strong></td>
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<td><strong>Timeline of L-DOPA treatment</strong></td>
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<td><strong>6-OHDA</strong></td>
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response) following L-DOPA injections, in which the animals were quiet, or even sleeping, were recorded daily. For a quantitative evaluation of the behavioral response, the first and the last L-DOPA injections were administered after the rats were introduced into circular arenas and their behavior was videotaped and subsequently quantified. The arenas comprised of flat transparent floors encircled by transparent Plexiglas cylinders 40 cm in diameter and 40 cm high. High-resolution microcameras (B/W CCD videocameras, 1/3 inch, VPC-465, CES AG, Switzerland) were located above and below the transparent base of the arenas. Once placed into the arena, the animals were allowed 10–15 min of habituation and were then treated with benzerazine and 30 min later injected with L-DOPA. The behavior in response to L-DOPA was videotaped for 2 h. Animals receiving a chronic treatment were sacrificed 2 h following the last L-DOPA or saline injection. Animals receiving acute injections were tested only once in the circular arenas, and were sacrificed 2 h following L-DOPA or saline injection.

The following scale was used to quantify the pattern of turning: 1, wide turns, usually along the walls or in circles with diameter at least half of the arena’s diameter; 2, tight turns, from head to tail; 3, very tight turns, typically rats would stand in a vertical posture with literally twisted trunk.

We quantified the development and gravity of dyskinetic movements which included: (a) vertical posture while turning, (b) axial twist of the body directed contralaterally to the lesioned side, (c) loss of balance, (d) repeated circulatory movements of the contralateral forelimb in front of the snouts and grabbing movements. Usually, up and down head movements, masticatory movements, licking, and sniffing accompanied turning throughout the session and were not quantified. Considering that these behaviors appeared to be tightly linked with each other and clearly distinguishable form turning, we combined the behavioral scores to form a single index of dyskinesias.

Histology and Immunohistochemistry

Two hours after the last L-DOPA or saline injection, rats were deeply anesthetized with Nembutal (100 mg/kg, ip) and then perfused with a cold solution of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4, 250 ml after clamping the descending aorta). Brains were removed and postfixed for 1–2 h in cold fixative and then transferred to a 30% sucrose solution overnight at 4°C. Brains were cut with a freezing microtome and transverse sections (40 μm thick) were collected throughout the rostrocaudal extent of the striatum, hypothalamus, and substantia nigra compacta (SNc). The placements of the 6-OHDA lesions were examined using cresyl violet staining. Using standard ABC methods, sections were processed for tyrosine hydroxylase (TH) to estimate the extension of 6-OHDA lesions and for c-fos immunoreactivity. Following rinses in PBS, free floating sections were blocked for 1 h in 5% normal goat or donkey serum plus 0.3% Triton X-100 to avoid background staining. Sections were then incubated overnight at 4°C with the primary antibodies sheep anti-TH (1:1000, Pel-Freez, Roger, AR), or rabbit anti-c-fos (1:4000, Oncogene Research Products, Calbiochem). Following a 1-h incubation at room temperature in biotinylated secondary antibodies (donkey anti-sheep and goat anti-rabbit, 1:300, Jackson ImmunoResearch, West Grove, PA) and a 1-h incubation with the ABC complex ( Vectastain Elite, Vector, Burlingame, CA), immunoreactivity was visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, Buchs, CH). Nickel chloride (0.08%) was added to the DAB solution to intensify the staining. Controls for nonspecific staining were performed in which either the first or the secondary antibody was omitted. These controls did not produce any staining.

Quantification and Analysis

To analyze the lesion placements, 5 to 6 brain sections were taken at the level of the MFB (AP −1.8 to −4.8). To estimate the extension of dopaminergic cells and loss of fibers, 4 brain sections were taken at the level of the SNc between AP −4.52 and −6.30 and 4 sections were taken at the level of the striatum between AP 1.60 and 0.20. For c-fos labeling, 4 to 5 sections were taken at the level of the striatum between AP 1.60 and 0.48. All the anterior-posterior (AP) coordinates were taken from Bregma as defined in Paxinos and Watson (1986). For all markers, labeled sections were examined using bright-field microscopy. Due to the complete loss of labeled cells in the SNc, TH-immunoreactive cell counting was not required. Cells labeled for c-fos were counted only in the striatum ipsilateral to the 6-OHDA lesions. For this purpose, the striatum was divided into four topographical areas encompassing dorsomedial (DM), dorsolateral (DL), ventromedial (VM), and ventrolateral (VL) regions and the number of labeled cells were counted within a fixed box (area = 500 × 500 μm) positioned approximately in the middle of these 4 striatal areas. For c-fos quantification, digitized bright-field images were captured using a Zeiss Axiophot microscope (10–100 × magnification) in combination with a Kodak Megaplus video camera (Eastman Kodak, San Diego, CA). The cell counting was done using the image analysis program Image-Pro Plus (Media Cybernetics, Silver Spring, MD). Photomicrographs of TH and c-fos were obtained using the system described above. Statistical evaluations for turning behavior were based on the analysis of variance (ANOVA) with turning rate as the behavioral-dependent measure.
ANOVA in the acute L-DOPA groups consisted of the between subjects (Ss) variable Group (5 groups injected only once with L-DOPA at 2, 3, 6, 10, or 18 weeks postlesion) and the within Ss variable Time post-L-DOPA (7 time periods of 5 min each starting at 5, 10, 15, 30, 60, 90, and 120 min after L-DOPA injection). For the chronic L-DOPA-treated groups, the analysis consisted of the between Ss variable Group (4 groups treated with L-DOPA for 1, 4, 8, or 16 weeks), the within Ss variables Session (first vs last L-DOPA treatment) and Time post-L-DOPA (7 time periods of 5 min each starting at 5, 10, 15, 30, 60, 90, and 120 min after L-DOPA injection). In order to compare the behavior of the chronic groups, on their last L-DOPA administration, to the behavior of the acute groups, we used the Group and Time post-L-DOPA variables, as described above, and the additional between Ss variable Treatment (chronic vs acute L-DOPA).

Turning pattern and intensity of dyskinetic movements were analyzed using nonparametric statistics. The Mann-Whitney test was used to analyze the effect of L-DOPA treatment (first vs last) for each single group (1, 4, 8, and 16 weeks, within subjects comparison). The Kruskal Wallis and the Wilcoxon signed rank tests were used to analyze differences between the 4 groups (1, 4, 8, and 16 weeks, between subject comparison) at the time of their first L-DOPA and at the time of their last L-DOPA treatment.

c-fos immunoreactivity in the acute and chronic L-DOPA groups was analyzed using ANOVA. For the acute groups, ANOVA consisted of the between Ss variable Group (4 groups injected only once with L-DOPA at 3, 6, 10, or 18 weeks postlesion, and the within Ss variable Striatal Area (4 striatal areas DM, DL, VL, VM, AP between 1.00 and 0.70). For the chronic groups, ANOVA consisted of the between Ss variable Group (4 groups treated with L-DOPA for 1, 4, 8, and 16 weeks, and the within Ss variable Striatal Area (4 striatal areas DM, DL, VL, VM). When the acute groups were compared to the chronic groups, the between Ss variable Treatment (chronic vs acute L-DOPA) was also used. Post hoc Fisher’s PLSD test was used when necessary. A P value < 0.05 was considered to represent a significant difference. All numerical data reported in the result section are given as mean ± SEM.

RESULTS

Since the control groups (saline-treated animals) were not affected behaviorally or anatomically they were not included in either the behavioral or the anatomical analysis.

Chronic L-DOPA Treatment for 1, 4, 8, or 16 weeks: Effects on Turning Rate

Two weeks after the lesion the first L-DOPA injection induced a short period of slow ipsilateral turning (in 28 out of 40 animals) which lasted for about 20–30 min (Fig. 1A). Subsequently, rats switched to contralateral turning that lasted throughout the 2 h of testing (Fig. 1B, first L-DOPA). On the subsequent days of treatment, ipsilateral turning was absent and the animals responded to L-DOPA only with contralateral turning, the rate of which increased during the first week of injections. Figure 1B shows an increase in contralateral turning rate, calculated as a number of turns/5 min, in response to the last vs the first L-DOPA treatment, which was confirmed by a significant session effect (F(1, 36) = 61.7, P < 0.001). Nevertheless, a separate ANOVA for the last L-DOPA treatment resulted in a nonsignificant group effect (F(3, 36) = 0.4, P = 0.76) indicating that the turning rate of the chronic groups was similar after 1 or 16 weeks of L-DOPA administration. This showed that the increase in turning rate, due to the chronic treatment, was already maximal after 1 week of L-DOPA treatment.

Turning Rate after an Acute L-DOPA Challenge Given at 2, 3, 6, 10, or 18 weeks Postlesion: Comparison with Chronic L-DOPA Treatment

Five groups of lesioned animals were treated with L-DOPA only once either on the day corresponding to the first L-DOPA administration (2 weeks postlesion) or at the time corresponding to the last L-DOPA administration in the chronic groups (3, 6, 10, or 18 weeks postlesion). Similarly to the chronically treated rats, in the acutely treated rats, L-DOPA administered at 2 or 3 weeks after the lesion induced a short period of ipsilateral turning (Fig. 2A). Thereafter rats switched to contralateral turning (Fig. 2B). When L-DOPA was administered for the first time at 6, 10, or 16 weeks postlesion, it induced only contralateral turning (Figs. 2A, B). The analysis of the contralateral turning rate showed a significant group × time post-L-DOPA interaction (F(24, 84) = 2.2, P < 0.005). Fisher post hoc confirmed a higher turning rate, within the first 15 min post-L-DOPA injection, in the groups treated at 10 and 18 weeks postlesion in comparison to the groups treated at 2, 3, or 6 weeks postlesion (P < 0.05). At the last time point, 120 min after L-DOPA, the groups treated at 6, 10, and 18 weeks postlesion showed higher turning rates in comparison to the group treated at 2 weeks postlesion (P < 0.01). Thus, contralateral turning in response to L-DOPA started faster and lasted longer when L-DOPA was administered at late time postlesion.

We considered it important to investigate whether the increase in turning rate due to length of time from the lesion, as shown in the acute L-DOPA groups, could also be expressed in the turning rate of the chronic L-DOPA groups. For instance, increase in turning rate on the last vs the first chronic L-DOPA injections could have been influenced by the time between the two
L-DOPA administrations. Thus, to assure that the increase in turning behavior was influenced mainly by repeated L-DOPA administration, we compared the turning rate of the chronic groups on their last L-DOPA injection with that of the acute groups at the corresponding time points postlesion. A highly significant treatment effect ($F_{1, 46}/H_{11005}/P_{11021} 17.2, P < 0.001)$ confirmed higher turning rates in the chronic vs the acute groups. This result showed that the increase in turning rate in the chronic L-DOPA groups was mainly due to the repeated L-DOPA treatment rather than to the elapsing of time between the first and the last L-DOPA administration (compare Fig. 1B with Fig. 2B).

**Dyskinetic Movements Induced by Chronic L-DOPA**

The dyskinetic movements were scored using the following scale: 0, absent; 1, infrequently present (mild); 2, present most of the time (or consistently present); 3, very strongly present, turning impeded. Figure 4 shows the development of dyskinetic movements in response to the first and last L-DOPA treatment in the chronic groups. The Mann-Whitney test showed that in all groups, dyskinetic movements at the last L-DOPA injection significantly differed from dyskinetic movements at the first L-DOPA injection (Fig. 4): 1 week $U = 1275, P < 0.001$; 4 weeks $U = 1407, P < 0.001$; 8 weeks $U = 1449, P < 0.001$; 16 weeks $U = 1283, P < 0.001$. The Kruskall-Wallis test showed that whereas dyskinetic movements were similar in all groups at the first L-DOPA injection, $H = 3.9, P > 0.3$, or at the last L-DOPA injection, $H = 2.7, P > 0.4$.

**FIG. 1.** Turning (mean ± SEM) induced by L-DOPA during the first and the last administration in chronically treated rats. L-DOPA was administered daily for 1, 4, 8, or 16 weeks. The first L-DOPA injection induced a short period of ipsilateral turning (A) that lasted 15–30 min. Thereafter rats turned only contralaterally to the lesion (B, first L-DOPA). The last L-DOPA injection (B) induced higher rates of contralateral turning compared to the first ($P < 0.001$), although, the temporal pattern of the response to L-DOPA did not visibly change over the two treatment schedules ($P = 0.76$).

**Pattern of Contralateral Turning in the Chronic Groups**

Following L-DOPA injection, the turning pattern was evaluated, using a score from 1 to 3, during 7 time points of 5 min each. Figure 3 shows the turning patterns after the first and after the last L-DOPA administration in the chronic groups. The Mann-Whitney test showed that in all groups, the pattern of turning at the last L-DOPA injection significantly differed from the pattern of turning at the first L-DOPA injection: 1 week $U = 1275, P < 0.001$; 4 weeks $U = 1407, P < 0.001$; 8 weeks $U = 1449, P < 0.001$; 16 weeks $U = 1283, P < 0.001$. The Kruskall-Wallis test showed that the groups did not differ from each other in the pattern of turning either at the first L-DOPA injection, $H = 3.9, P > 0.3$, or at the last L-DOPA injection, $H = 2.7, P > 0.4$. The dyskinetic movements were scored using the following scale: 0, absent; 1, infrequently present (mild); 2, present most of the time (or consistently present); 3, very strongly present, turning impeded. Figure 4 shows the development of dyskinetic movements in response to the first and last L-DOPA treatment in the chronic groups. The Mann-Whitney test showed that in all groups, dyskinetic movements at the last L-DOPA injection significantly differed from dyskinetic movements at the first L-DOPA injection (Fig. 4): 1 week $U = 1299, P < 0.001$; 4 weeks $U = 1155, P < 0.001$; 8 weeks $U = 989, P < 0.001$; 16 weeks $U = 1005, P < 0.001$. The Kruskall-Wallis test showed that whereas dyskinetic movements were similar in all groups at the first L-DOPA injection, $H = 5.7, P > 0.13$, they differed between groups at the last
L-DOPA injection, $H = 22.5$, $P < 0.0001$. The Wilcoxon signed rank test analysis for the last L-DOPA injection showed that the 1 week group differed from the other groups, $P < 0.001$, the 4 weeks group differed from the 16 weeks group, $P < 0.03$, but not from the 8 weeks group, $P = 0.3$; the latter was almost significantly different from the 16 weeks group, $P = 0.08$. These findings indicated that the increase in dyskinetic movements occurs during the first week of treatment with a further increase during the subsequent 15 weeks of treatment (see Fig. 4).

**Absence of Response to L-DOPA after Repeated Injections**

In an unpredicted manner some animals (up to 5 out of 10 per group) would not respond to the daily dose of L-DOPA. It was often the case that rats that had “no”

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**FIG. 2.** Turning (mean ± SEM) after a single L-DOPA challenge given at 2, 3, 6, 10, or 16 weeks postlesion. Ipsilateral turning was present only when L-DOPA was given at 2–3 weeks postlesion (A), and only contralateral turning was shown thereafter (B). As the time postlesion increased, the animals had the tendency to turn for longer periods after L-DOPA.

**FIG. 3.** The pattern of turning after the first and last L-DOPA injection was scored using the following scale; 1, wide turns; 2, tight turns, 3, very tight turns. Initially wide turning became tighter after about 30 min post injection, which also corresponded to the peak of L-DOPA-induced turning. Turns were tighter after the last compared to the first L-DOPA administration ($P < 0.001$).

**FIG. 4.** Intensity of dyskinetic movements during the first and the last L-DOPA administration in chronically treated rats. Chronic L-DOPA administration induced an increase in the presence of dyskinetic movements already after 1 week of treatment ($P < 0.001$). Prolonged L-DOPA treatment further increased dyskinetic movements especially in the 16 weeks group ($P < 0.001$).
response to L-DOPA would have recurrent “no” response episodes. This was especially evident in the groups treated for 8 and 16 weeks where the same animals had “no” response to L-DOPA for 2–3 days in a row for a total of 10 or even 20 episodes during the whole treatment. We did not perform statistical analysis on these data.

Location and Extension of the Lesion: Tyrosine Hydroxylase Immunolabeling

The animals constituted a rather homogeneous population in terms of extension of the dopaminergic lesions and therefore none of them were excluded from the study. Histological examination revealed that the site of 6-OHDA injections was located at the level of the MFB (AP = 3.3–4.3 posterior to Bregma). In 3 animals the lesion was placed at the level of the substantia nigra compacta (SNc). Nevertheless, their behavioral expressions and anatomical outcome (TH labeling and c-fos expression) were comparable to those of the corresponding groups and they were therefore included in the study. The unilateral 6-OHDA lesions resulted in a severe decrease in TH immunolabeling in the side ipsilateral to the lesion when compared to the contralateral side (Fig. 5). In all animals the SNc was devoid of TH-labeled cells except for a few labeled neurons (n = 2–3) detected in the most medial parts. As a consequence of dopaminergic cell loss, the ipsilateral striatum was completely deficient of dopaminergic terminals. This was revealed by a complete absence of striatal TH immunolabeled fibers (Fig. 5). The lateral part of the VTA was also affected and as a result there was a decrease in TH labeling in the ipsilateral nucleus accumbens, septum, olfactory tubercle, and cortical areas, compared to the intact side.

The Expression of c-fos in the Lesioned Striatum: Effects of Acute and Chronic L-DOPA Treatment

In the striatum-depleted dopaminergic terminals acute and chronic L-DOPA induced extensive c-fos expression. After L-DOPA, only a few cells were labeled for c-fos in the striatum contralateral to the lesion (Fig. 6), or bilaterally after saline treatment. Thus, the analysis for c-fos was performed only in the lesioned striatum of rats treated with L-DOPA. In the acute groups, c-fos-labeled cells were present throughout the striatum (Fig. 7) with the exception of the acute group treated for the first time with L-DOPA at 10 weeks after the lesion. This group unexpectedly showed low c-fos labeling (Fig. 7C). This group was excluded from c-fos analysis. ANOVA showed a nonsignificant group effect (F 2, 9 = 1.7, P = 0.23), indicating that the ability of a single injection of L-DOPA to induce c-fos expression in the lesioned striatum was not influenced by the time postlesion (Fig. 9B).

After chronic treatment, L-DOPA was less effective in inducing c-fos expression compared to an acute treatment (compare Fig. 7 to Fig. 8). Reduction in c-fos expression in the chronic vs the acute groups was confirmed by a significant treatment effect (F 1, 47 = 12.8, P < 0.001), (Figs. 9B, C). A separate ANOVA of the chronic groups revealed a nonsignificant group effect (F 3, 35 = 1.4, P = 0.25), indicating that after 1 week of treatment L-DOPA did not further reduce c-fos expression. In addition, a significant area effect (F 3, 105 = 73.9, P < 0.001) indicated that the decrease in the number of c-fos-labeled cells after chronic L-DOPA treatment was area specific. This involved, to a great extent, the DM, VM, and VL, and to a lesser extent, the DL area of the striatum (Fig. 9C).

c-fos Expression and Its Relation to Behavior

As shown earlier, chronic L-DOPA increased the behavioral response in all treated groups. In the same animals striatal c-fos expression decreased compared to animals treated with acute injections. Correlations were run to relate the behavioral response, turning, and dyskinesias, to the regional expression of c-fos in the striatum of chronically treated rats. All the chronic groups were used in the analysis independently of the

FIG. 5. Photomicrographs showing representative transverse sections of the striatum (Str, A) and the substantia nigra (SNc, B) of rats with unilateral lesions labeled with TH. Note the complete loss of TH immunoreactivity in the Str (A) and in the SNc (B) in the lesioned side compared to the nonlesioned side (arrows). The lesion also affected the ventral tegmental area (VTA), the nucleus accumbens (Acb), and the tubercle olfactorium (TbO). cc, corpus callosum; ctx, cortex.
length of L-DOPA treatment. The values used to express turning behavior and dyskinesias were the total number of turns quantified at 7 time periods of 5 min each, and the maximal score of dyskinesias during the 2-h session. Moreover, considering that we found regional differences in c-fos expression (see previous section) and that the dorsolateral striatum is preferentially associated with limb and axial movements (7), possibly contributing to the dyskinetic behavior (10), the number of c-fos-labeled cells was expressed by calculating a dorsolateral (dl) index as follows: \( \text{dl} = \frac{\text{DL}}{\text{DM} + \text{VL} + \text{VM}} \). This index expresses the ratio of c-fos-labeled cells in the dorsolateral region over the rest of the striatum. The analysis showed that the c-fos index dl was negatively correlated with total turning \((r = -0.36, F_{1,38} = 5.5, P < 0.03)\), and positively correlated with the maximal score of dyskinesias \((r = 0.31, F_{1,38} = 4.04, P = 0.05)\).

**DISCUSSION**

It is commonly observed that in the majority of PD patients unwanted side effects develop after extensive periods of treatment with L-DOPA. We addressed this issue by examining the progression of behavioral sensitization to L-DOPA in a rat model of PD. We measured turning behavior and the development of dyskinetic movements induced by L-DOPA treatment over a period of 16 weeks. Our results showed that behavioral sensitization to L-DOPA, as expressed by increase in turning behavior and changes in its rate and pattern, is initiated from the very first exposure to the drug, reaches its maximum expression after 1 week, and continues to stay high during the whole L-DOPA treatment without further development. Along with increase in turning rate, dyskinesias also develop during the first week of treatment and seem to increase further during the 16 weeks of L-DOPA treatment.

We showed that the very first L-DOPA administration induced a weak transitory ipsilateral turning that was replaced by a slow but consistent contralateral turning behavior. This switch, from ipsilateral to contralateral turning, provides the first sign of increased behavioral responsiveness of the lesioned striatum taking place already within the first L-DOPA session. This

**FIG. 6.** Photomicrographs showing c-fos expression in the whole striatum after acute (A) and chronic (B) L-DOPA treatment. Note the almost complete absence of c-fos expression in the intact (left) compared to the lesioned (right) side of the striatum. Calibration bar = 150 μm.
could be explained by an increase in extracellular DA levels in the lesioned striatum, compared to the nonlesioned striatum (5, 18), due to increased L-DOPA metabolism from different striatal sources (28, 34, 52) and/or increased release of DA from a few surviving terminals (29). Besides, behavioral changes may follow even after small increases in extracellular DA, as the released DA may activate supersensitive DA receptors in the lesioned striatum (21, 26, 54). Our results showed that repeated treatment with L-DOPA not only induced an increase in the rate of contralateral turning, but also had a strong effect on the pattern of turning. Wide turns, usually present during the first day of treatment with L-DOPA, became tighter already after 1 week of treatment and did not change thereafter, suggesting a parallel development with the intensity of turning. Overall, these results point out that L-DOPA induces behavioral sensitization from the first administration, as shown by changes in the direction, intensity, and pattern of turning. Sensitization to L-DOPA is also supported by the development and progress of dyskinetic movements throughout the 16 weeks of treatment. Over the weeks turning induced by L-DOPA was often interrupted, sometimes up to minutes, due to the overwhelming interference of dyskinetic movements that involved the whole body of the animals (9). Once turning was reinstated as the main behavior performed by the rats, it had a burst pattern with particularly high turning rates that compensated, to some extent, for the reduced turning during periods of intense dyskinesias. It is possible that a lack of change in the overall turning rate after the first week of treatment is related to the development of excessive dyskinetic movements, which competed with turning for the overall motor output.

The mechanisms underlying L-DOPA-induced excessive behavioral outcome could be related to changes in the activity of striatal DA receptors. However, it is unclear how and which DA receptor subtypes, D1 vs D2 (16, 37) or D3 (4), are preferentially involved. Studies...
in rats have demonstrated that changes in DA receptor responsiveness, induced by repeated L-DOPA, are tightly related to changes in neuropeptides content (16, 40, 51). In this respect it has been shown that repeated L-DOPA administration in the rat model of PD is accompanied by elevations in dynorphine precursor in the striatonigral system (9, 16, 20). Given the inhibitory effect of dynorphin in SNr (27), the elevation in striatonigral dynorphin after L-DOPA may decrease the activity of the GABAergic SNr output neurons with consequent facilitation of movements (27). Accordingly, Cenci et al. (10) have shown that L-DOPA-induced dyskinetic movements in rats are directly correlated with increased dynorphin levels in striatonigral neurons. Increased dynorphin function in the SNr could possibly mediate the behavioral sensitization to L-DOPA as expressed by the development of uncontrolled movements such as dyskinesias.

The expression of the immediate-early gene c-fos is considered a marker for neuronal activation, and L-DOPA induces the expression of c-fos in the striatum ipsilateral to the lesion, most likely through mechanisms that reflect postsynaptic DA D1 or D1/D2 receptor stimulation (3, 15, 17, 32, 38, 46). Our results showed that the ability of L-DOPA to induce c-fos in the DA denervated striatum was reduced after 1 week of L-DOPA treatment when compared to an acute treatment. Nevertheless, there was a persistent c-fos expression in the dorsolateral regions of the striatum throughout the 4 months of treatment. We reasoned that persistent c-fos expression in the dorsolateral regions of the striatum, reflecting neuronal activation induced by chronic L-DOPA treatment, could be associated with the development of dyskinesias. Indeed, our results support this speculation as we found a positive significant correlation between the highest score of dyskine-

**FIG. 8.** Photomicrographs showing c-fos expression in the lesioned side of the striatum after chronic L-DOPA given for 1 week (A), 4 weeks (B), 8 weeks (C), and 16 weeks (D). Note the preferential distribution of labeled cells in the dorsolateral regions in all groups. Calibration bar = 100 μm.
Moreover, we found a negative correlation between c-fos in the dorsolateral striatum and turning, which is in line with the idea that turning and dyskinesias compete for the overall motor output, i.e., when dyskinesias exceed a certain level turning behavior is no longer carried out.

It is possible that mechanisms underlying the expression of c-fos after chronic L-DOPA treatment differ between striatal regions (10, 49). Selective expression of c-fos in specific striatal regions was speculated to be dependent on the level of postsynaptic kappa opioid receptors involved in the regulation of DA input to striatonigral neurons (51). For instance, after 6-OHDA lesions chronic dopamine D1 receptors stimulation was found to reduce striatal c-fos expression except in the dorsolateral striatum (using SKF 38393). The persistency of c-fos in the dorsolateral regions was explained by the loss of the inhibitory action of dynorphin in the dorsolateral striatum that requires dopamine terminals (presynaptic kappa receptors), which are eliminated in the rat model of PD (51). A similar mechanism could explain our results on c-fos expression after chronic L-DOPA stimulation. The expression of another early gene, fosB, whose induction also depends upon D1 receptors activity, has been found to change after altered dopaminergic neurotransmission (14). Increase in fosB expression was present mainly in dorsolateral regions of the striatum and was associated with the presence of L-DOPA-induced dyskinesias in the rat (2). Here it is worth noting that the number and distribution of c-fos-positive cells we observed in the lesioned striatum, after 1 week of chronic L-DOPA, were similar to the number and distribution of fosB-positive cells reported by Cenci et al. (10). Overall, changes at the neuronal level induced by chronic L-DOPA are mainly region specific and involve mainly the dorsolateral striatum, which is known to be the area where the limbs are highly represented (7, 13, 55).

In conclusion, our results suggest that changes induced by L-DOPA, in the dopamine-depleted rat, eventually leading to side effects, take place already after the very first challenge. Furthermore this study supports the unilateral DA-depleted rat model as a suitable model to study L-DOPA-induced dyskinesias. Regional changes in c-fos expression, especially marked in dorsolateral areas, highlight heterogeneity in the L-DOPA mechanism of action within the striatal circuitry. Persistent c-fos expression in dorsolateral areas of the striatum may be related to the development of L-DOPA-induced dyskinetic movements in rats.

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