L-Dopa restores striatal dopamine level but fails to reverse MPTP-induced memory deficits in rats

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Abstract

The objective of the present investigation was to test the effects of benserazide/L-dopa treatment in a model of learning and memory deficits associated with early Parkinson’s disease. Intra-nigral administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) caused a lesion in the substantia nigra, compact part and a specific loss of dopamine (DA) and its metabolites in the striatum of rats and a memory impairment in the two-way active avoidance task. The administration of benserazide/L-dopa (50 and 200 mg/kg) to the MPTP-lesioned rats restored the striatal level of DA, but did not reverse the MPTP-induced learning and memory impairment. As this treatment caused a large increase of DA levels in extrastriatal brain regions of the MPTP-lesioned animals, this study suggests that benserazide/L-dopa therapy was not effective in improving the observed learning impairment because this treatment appears to tilt the balance between DA levels in the striatum and in the extrastriatal regions, such as frontal cortex and limbic structures, resulting in a cognitive deficit.

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Key words: Dopamine, L-dopa, memory, MPTP, Parkinson’s disease, striatum.

Introduction

L-Dopa, a precursor of dopamine (DA) that is converted to DA in the brain, is the most effective drug used to reverse the motor impairments observed in Parkinson’s disease (PD) patients and also in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey and mouse models of PD (Obeso et al., 2000). However, the beneficial effect of L-dopa in improving the cognitive function affected in PD is controversial. While earlier studies indicated an improvement of cognitive functions in PD patients treated with L-dopa (Beardsley and Puletti, 1971; Loranger et al., 1972), other studies have shown that this treatment may cause only mild improvements (Crowdon et al., 1998; Pillon et al., 1989), or even aggravate PD cognitive impairments (Huber et al., 1989; Poewe et al., 1991; Prasher and Findley, 1991).

Systemic administration of MPTP causes acute and irreversible Parkinson-like symptoms predominantly in man and monkeys (Bezard et al., 1998). A preliminary report from our laboratory suggested that the significant learning deficit observed in rats that receive intra-nigral administration of MPTP and later submitted to the two-way active avoidance tasks may be considered a model of learning and memory deficits observed in PD (Da Cunha et al., In Press). This model is particularly useful to evaluate learning and memory alterations dependent upon neural circuits affected in PD since a marked and selective lesion in the dopaminergic neurons of the substantia nigra, compact part (SNC) is observed in these animals and they present no gross motor impairment that would confound interpretation of the impaired learning scores (Da Cunha et al., In Press).

Taking these considerations into account, the present investigation addressed the effects of L-dopa treatment in this rat model of PD-related learning and memory deficits. Besides testing the effectiveness of this treatment in a model in which specific dopaminergic pathways are selectively affected, the impact of the treatment upon the alteration in the content of DA and other monoamines was also assayed in the striatum and other DA-innervated structures.
Material and methods

Subjects

Male Wistar rats from our own breeding stock weighing 250–300 g at the beginning of the experiments were used. The animals were maintained in a temperature-controlled room (22 ± 2 °C) on a 12/12 h dark/light cycle (lights on 07:00 hours) with food and water available ad libitum. All the behavioural experiments were conducted between 12:00 and 18:00 hours, and were in accordance with the accepted ‘Guidelines of the principles of the care and use of animals’ of our Institution. The animals were maintained in Plexiglas home cages (60 × 25 × 25 cm) throughout the tests.

Surgery

Twenty-three animals each received atropine sulphate (0.4 mg/kg, i.p.) to suppress salivation and penicillin G-procaine (20 000 U in 0.1 ml, i.m.) and were anaesthetized with sodium thiopental (40 mg/kg, i.p.). MPTP HCl (1 µmol in 2.1 µl of saline, 0.35 µl/min, Sigma Chemical Co.) was bilaterally infused through a 30-gauge needle according to the following coordinates: anteroposterior (AP), −5.0 mm from bregma; mediolateral (ML) ± 2.1 mm from midline; dorsoventral (DV), −7.7 mm from skull, adapted from Paxinos and Watson (1986). The injection needle was retained in place for an additional 2 min to maximize diffusion of the solution. After surgery the animals remained in a temperature-controlled box until they recovered from anaesthesia. The animals were then returned to their home cages and allowed to recover for 20 d after surgery before the beginning of training.

Post-surgery drug administration procedures

The animals were divided into 4 groups:

Group 1: Non-operated animals that received vehicle (12 animals).
Group 2: Non-operated animals that received benserazide/l-dopa (12 animals).
Group 3: MPTP-lesioned animals that received vehicle (11 animals).
Group 4: MPTP-lesioned animals that received benserazide/l-dopa (12 animals).

Benserazide (Sigma Chemical Co.) was dissolved in saline and l-dopa (Sigma Chemical Co.) was dissolved in the following vehicle: 20% Alkamus El-20 (Rhône-Poulenc, USA) and 80% saline. Benserazide (50 mg/kg) and l-dopa (200 mg/kg) or their respective vehicles were administered i.p. 45 min and 30 min before training in the two-way active avoidance task and before the animals’ decapitation. The doses of drugs used in the present study were chosen from a dosage range reported in the literature (Harik et al., 1987; Menzaghi et al., 1997).

The two-way active avoidance task

The active avoidance test apparatus was an automated 23 × 50 × 23 cm shuttle-box (Gemini Avoidance System, San Diego Instruments, San Diego, CA, USA) with a dark front glass and a floor made of parallel 5 mm calibre stainless-steel bars spaced 15 mm apart. The box was divided into two compartments of the same size by a wall with a door that remained open during the tests. In the training session, after 3 min of habituation, 30 sound cues (conditioned stimulus: 1.5 kHz, 60 dB, maximum duration of 5 s) were paired with a subsequent 0.5 mA footshock (unconditioned stimulus: maximum duration of 5 s) until the animal crossed to the other compartment. The animal could avoid the shock by crossing to the other side during the presentation of the conditioned stimulus (active avoidance). The time between each conditioned stimulus presentation varied randomly, ranging from 10 to 50 s. The number of active avoidances, the latency to cross to the other side of the box after the beginning of each conditioned stimulus (escape latency), and the number of inter-trial crossings between the two box compartments were recorded automatically by the apparatus. The test session, conducted 24 h later, was identical to the training one, except for a 1 min habituation time (for more details, see Da Cunha et al., In Press).

Determination of striatal monoamine levels

One month after the behavioural test, 8 animals of each group received the corresponding benserazide and l-dopa treatment and were sacrificed by decapitation. Their striatum, frontal cortex, amygdala, hippocampus and nucleus accumbens were rapidly dissected on dry ice and stored at −70 °C until the determination of monoamine levels. The levels of noradrenaline (NA), DA, serotonin (5-HT), and their non-conjugated metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilliac acid (HVA), 5-hydroxy-indoleacetic acid (5-HIAA), and l-dopa were assayed by reverse-phase high performance liquid chromatography with electrochemical detection, as described by Hallman and Jonsson (1984). Briefly, a C18 reverse phase column (Shim-pack, CLC-ODS 150 × 4.6 mm, Shimadzu), an amperometric detector (Shimadzu, L-ECD-6A) and a liquid chromatography
series of 30-µm formalin. The brains were then removed and placed parts of the dopaminergic cell groups that preserved a animals, and then compared them to the areas of uninjured sections in millimetres. Thus, we first determined the areas it always corresponded to the real dimensions of the brain objectives, the scale of the drawings was adjusted so that microscope aimed at a flat screen computer monitor combined with a drawing tube on the Leitz Diaplan and thionin-stained sections using a commercially avail-
determined from tyrosine hydroxylase-immunostained architectural purposes. 

Histology and lesion analysis

In order to evaluate the extent of the midbrain dopaminergic cell damage induced by the MPTP dose currently used we performed histological analysis in another group of 9 MPTP-treated animals and 8 control non-operated animals. The animals were deeply anaesthetized with 200 mg/kg thiopental and perfused transcardially with 150 ml of 0.9% NaCl followed by 500 ml of 10% formalin. The brains were then removed and placed overnight in the perfusate with 20% sucrose added. Four series of 30-µm thick sections were cut on a sliding microtome in the frontal plane and collected from the caudal diencephalon to the caudal midbrain. One series was immunostained for tyrosine hydroxylase with a monoclonal antibody to tyrosine hydroxylase raised in mice (1:5000 dilution; Incstar). The antigen–antibody complex was localized using a variation of the ABC system with a commercially available kit (ABC Elite kit, Vector Laboratories). Slides were then dehydrated and coverslipped with DPX. An adjacent series was stained with thionin to serve as a reference series for cytoarchitectural purposes.

The area of the midbrain dopaminergic cell groups was determined from tyrosine hydroxylase-immunostained and thionin-stained sections using a commercially available computer drawing program (Auto Cad, release 12) combined with a drawing tube on the Leitz Diaplan microscope aimed at a flat screen computer monitor (Zenith VGA, model 1492). To allow use of multiple objectives, the scale of the drawings was adjusted so that it always corresponded to the real dimensions of the brain sections in millimetres. Thus, we first determined the areas of the midbrain dopaminergic cell groups in control animals, and then compared them to the areas of uninjured parts of the dopaminergic cell groups that preserved a normal cytoarchitectural appearance and tyrosine hydroxylase immunostaining in MPTP-treated animals. For both experimental groups, tyrosine immunoreactive cells of the midbrain dopaminergic groups (i.e. SNc, ventral tegmental area (VTA), and retrorubral field) were plotted using a Nikon Optiphot-2 microscope equipped with a camera lucida, and counted from an entire series of sections collected at 150 µm intervals.

Statistics

Neurochemical data were analysed by two-way analysis of variance (ANOVA). Data of the ambulatory behavior (inter-trial crossings) were analysed by two-way ANOVA. Active avoidance data were analysed by three-way ANCOVA with MPTP treatment as one factor, L-dopa treatment as the second factor (independent factors) and the session day as the third factor (repeated measure). In this analysis the number of avoidances was the dependent variable and the number of inter-trial crossings was the covariate. Differences between groups were further analysed by the post-hoc Duncan test. Differences were considered to be statistically significant when $p \leq 0.05$.

Results

The intra-SNc, MPTP microinfusion produced an area of neuronal cell loss filled with gliosis as well as a reduction in tyrosine hydroxylase-immunostained cells in the SNc with minor lesions in the surrounding areas (see Figure 1 and Table 1). The parameters described above for MPTP lesions resulted in a lesion involving 67% of the SNc. In addition, MPTP lesions also spread to a lesser extent (17%) to the VTA and to the retrorubral field (about 32%) (Table 1). The number of tyrosine immunoreactive cells is also presented in Table 1.

The effects of MPTP lesion and benserazide/L-dopa treatment on the cerebral levels of DA, 5-HT, NA and their non-conjugated metabolites (DOPAC, HVA and 5-HIAA) are illustrated in Table 2. As can be seen, the MPTP lesion caused a significant reduction of about 41% in striatal levels of DA compared to controls, without altering the DA levels in other extrastriatal brain structures (in order to avoid redundancy in the text, the ANOVA values for the neurochemistry data have been omitted; $p \leq 0.05$, Duncan test). No significant MPTP lesion-induced alteration was observed in the levels of NA, 5-HT and 5-HIAA in any of cerebral structures studied ($p \geq 0.05$, Duncan test). Treatment of MPTP-lesioned animals with benserazide/L-dopa replaced the normal level of DA in the striatum since there was no
Figure 1. Bright-field photomicrographs of a thionin-stained (A, B) and an adjacent tyrosine hydroxylase-immunostained (A’, B’) section illustrating the appearance of a control (A, A’) and a MPTP-lesioned animal (B, B’). Scale bar = 500 µm. fr, Fasciculus retroflexus; SNc, substantia nigra, compact part; SNr, substantia nigra, reticular part; VTA, ventral tegmental area.

Table 1. Lesion in the midbrain dopaminergic cells of rats due to the intra-nigral administration of 1 µmol MPTP

<table>
<thead>
<tr>
<th>Number of TH immunoreactive cells</th>
<th>MPTP-lesioned area(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group(^a)</td>
<td>MPTP-treated animals(^a)</td>
</tr>
<tr>
<td>SNc (A9)</td>
<td>4378.7 ± 247.9</td>
</tr>
<tr>
<td>VTA (A10)</td>
<td>3692.4 ± 154.2</td>
</tr>
<tr>
<td>RRN (A8)</td>
<td>762.5 ± 28.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M.

\(^a\) Mean number of tyrosine immunoreactive cells in the midbrain dopaminergic groups of control and MPTP-treated animals.

\(^b\) Lesioned area filled with gliosis and reduction in tyrosine hydroxylase-immunostained cells (as seen in Figure 1B’).

SNc, substantia nigra, compact part; VTA, ventral tegmental area; RRN, retrorubral field.
Table 2. Effect of the administration of 1 μmol MPTP into the rat SNc on the striatal levels of monoamines

<table>
<thead>
<tr>
<th>Concentration (ng/g wet tissue)</th>
<th>Striatum</th>
<th>Cortex</th>
<th>Amygdala</th>
<th>Hippocampus</th>
<th>N. accumbens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DA</td>
<td>DOPAC</td>
<td>HVA</td>
<td>5-HT</td>
<td>5-HIAA</td>
</tr>
<tr>
<td>Control/vehicle</td>
<td>5333.13±319.13</td>
<td>1490.15±129.73</td>
<td>298.56±21.40</td>
<td>257.66±10.64</td>
<td>548.72±20.29</td>
</tr>
<tr>
<td>MPTP/vehicle</td>
<td>3150.26±371.43*</td>
<td>1018.54±80.78*</td>
<td>219.92±21.50*</td>
<td>283.33±9.52</td>
<td>550.08±23.58</td>
</tr>
<tr>
<td>Control/l-dopa</td>
<td>15260.91±611.90*#</td>
<td>3955.92±255.07##</td>
<td>644.18±54.48*#</td>
<td>223.07±16.99#</td>
<td>614.17±56.38</td>
</tr>
<tr>
<td>MPTP/l-dopa</td>
<td>5368.08±597.47##</td>
<td>3184.90±378.83*#</td>
<td>444.51±44.50*#</td>
<td>162.90±8.70*#</td>
<td>554.87±45.13</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>44.47±7.48</td>
<td>39.62±8.39</td>
<td>23.41±8.16</td>
<td>354.78±41.79</td>
<td>339.58±21.22</td>
</tr>
<tr>
<td>MPTP/vehicle</td>
<td>21.85±3.85</td>
<td>16.33±1.41</td>
<td>11.27±1.39</td>
<td>306.31±82.14</td>
<td>339.55±21.81</td>
</tr>
<tr>
<td>Control/l-dopa</td>
<td>468.58±48.15**#</td>
<td>528.23±41.44**#</td>
<td>123.42±8.87*#</td>
<td>229.59±35.83</td>
<td>384.35±28.50</td>
</tr>
<tr>
<td>MPTP/l-dopa</td>
<td>605.26±93.66**#</td>
<td>532.52±75.20**#</td>
<td>134.75±37.38**#</td>
<td>253.02±71.81</td>
<td>384.09±25.84</td>
</tr>
<tr>
<td></td>
<td>99.78±10.38</td>
<td>63.07±8.86</td>
<td>10.50±1.53</td>
<td>382.21±24.81</td>
<td>523.09±16.56</td>
</tr>
<tr>
<td>MPTP/vehicle</td>
<td>69.21±6.03</td>
<td>49.12±2.18</td>
<td>13.51±2.24</td>
<td>309.78±23.53*</td>
<td>523.33±39.49</td>
</tr>
<tr>
<td>Control/l-dopa</td>
<td>764.22±37.42**#</td>
<td>819.41±66.96**#</td>
<td>148.87±18.50**#</td>
<td>244.59±19.78*</td>
<td>577.84±34.78</td>
</tr>
<tr>
<td>MPTP/l-dopa</td>
<td>832.37±103.02**#</td>
<td>1039.47±183.85**#</td>
<td>141.93±19.77**#</td>
<td>261.78±18.52**</td>
<td>561.91±42.71**</td>
</tr>
<tr>
<td></td>
<td>14.27±0.84</td>
<td>11.37±2.18</td>
<td>6.30±1.21</td>
<td>200.72±21.82</td>
<td>422.42±38.27</td>
</tr>
<tr>
<td>MPTP/vehicle</td>
<td>16.83±2.80</td>
<td>15.06±3.01</td>
<td>5.65±1.13</td>
<td>245.31±18.21</td>
<td>342.13±16.83</td>
</tr>
<tr>
<td>Control/l-dopa</td>
<td>573.26±40.38**#</td>
<td>591.39±37.08**#</td>
<td>126.37±7.31**#</td>
<td>172.54±19.91**#</td>
<td>511.31±25.88**#</td>
</tr>
<tr>
<td>MPTP/l-dopa</td>
<td>752.29±76.01**#</td>
<td>568.18±88.84**#</td>
<td>124.55±4.17**#</td>
<td>181.11±18.01**#</td>
<td>478.7±21.54**#</td>
</tr>
</tbody>
</table>

The values represent the mean ± s.e.m. of 8 rats. *p ≤ 0.05 compared to the control/vehicle group; #p ≤ 0.05 compared to the MPTP/vehicle group; two-way ANOVA followed by Duncan test.
significant difference in the striatal DA level between this group and the non-operated animals treated with vehicle (p ≥ 0.05, Duncan test). The striatal levels of DOPAC and HVA in the MPTP/L-dopa group were significantly increased (p ≤ 0.05, Duncan test), indicating that part of the L-dopa (possibly taken up by the striatal neurons and metabolized to DA) was further metabolized to these substances.

The benserazide/L-dopa treatment per se increased the levels of DA, DOPAC, HVA, 5-HT, 5-HIAA, and NA in almost all the structures assayed compared to the non-operated animals receiving vehicle (Table 2). Thus, in the striatum and in the nucleus accumbens there was an increase in the levels of DA, DOPAC, HVA and NA (p ≤ 0.05, Duncan test). A significant increment in the levels of DA, DOPAC and HVA was observed in the frontal cortex and in the amygdala. Besides, in the amygdala there was also an increase in the levels of 5-HT (p ≤ 0.05, Duncan test). Also, a significant increment in the levels of DA, DOPAC, HVA, and 5-HT was observed in the hippocampus (p ≤ 0.05, Duncan test).

The treatment with benserazide/L-dopa also altered the levels of cerebral monoamines of the MPTP-treated group compared to the control/vehicle group (Table 2). In the striatum, besides the increase in the levels of DOPAC and HVA described above, we observed an increase in the levels of 5-HT and NA (p ≤ 0.05, Duncan test). The contents of DA, DOPAC, and HVA were significantly increased in the frontal cortex and hippocampus (p ≤ 0.05, Duncan test). In the amygdala there was a significant increase in the levels of DA, DOPAC, HVA, and 5-HT (p ≤ 0.05, Duncan test). Also, an increment in the levels of DA, DOPAC, HVA, and NA was observed in the accumbens (p ≤ 0.05, Duncan test, Table 2).

At the beginning of the memory tests (20 d after surgery) no gross behavioural changes were noted in the lesioned rats. The ambulatory behaviour of the animals during the training session of the two-way active avoidance task, evaluated by the number of inter-trial crossings, is presented in Figure 2. Two-way ANOVA followed by the Duncan test showed no difference between the scores of the MPTP-lesioned and non-lesioned rats [F(1,43) = 0.03; Duncan test, p ≥ 0.2]. On the other hand, benserazide/L-dopa treatment caused an increase of about 50% in the ambulation score [ANOVA, F(1,43) = 4.91; Duncan test, p ≤ 0.05] independent of the MPTP lesion [F(1,43) = 0.01; Duncan test, p ≥ 0.2].

The effects of the administration of benserazide/L-dopa (50 mg/kg; 200 mg/kg) to the MPTP-lesioned rats on the two-way active avoidance task are presented in Figure 3. These data were analysed by three-way ANCOVA considering the session day as a repeated measure and the treatment with MPTP and benserazide/L-dopa as independent variables and the inter-trial crossings as the covariate. This analysis ruled out the possibility that the stimulating effect of L-dopa on rat locomotion would affect the results reported below. This analysis showed an increase in the avoidance scores in the test session compared to the training session in the control.
non-operated animals receiving vehicle [ANOVA, \( F(1,43) = 24.52; \) Duncan test, \( p < 0.05 \)] or benserazide/l-dopa (\( p < 0.05, \) Duncan test). This result show that the non-leisioned animals learn to avoid the footshock and can remember it 24 h after a training session. On the other hand, the MPTP-lesioned rats could not significantly increase their avoidance scores in the test session compared to the training session (\( p > 0.2 \)), even after receiving benserazide/l-dopa before the training trial (\( p = 0.10 \)). In the training session, the MPTP-lesioned rats presented 50% fewer avoidance scores compared to the control non-operated rats [\( F(1,42) = 12.50; \) Duncan test, \( p < 0.05 \)]. The treatment with benserazide/l-dopa did not reverse this deficit – a 78% lower score was observed in this group compared to the non-operated control animals (ANOVA, \( F(1,42) = 14.89; \) Duncan test, \( p < 0.05 \)). No significant difference was observed between MPTP-lesioned rats receiving vehicle or benserazide/l-dopa in the training session (\( p > 0.2, \) Duncan test). The administration of benserazide/l-dopa by itself caused a 56% decrease in avoidance score in the training session compared to the non-operated animals receiving vehicle (\( p < 0.05, \) Duncan test). In the test session, the number of avoidance of the MPTP-lesioned rats was 62% lower than that of the control non-operated rats (\( p < 0.05, \) Duncan test). Treatment with benserazide/l-dopa did not reverse this deficit since a 68% lower score was observed in this group compared to the non-operated control animals in the test session (\( p < 0.05, \) Duncan test).

No significant difference was observed between MPTP-lesioned rats receiving vehicle or benserazide/l-dopa in the test session (\( p > 0.2, \) Duncan test). Taken together, these results show that treatment with benserazide/l-dopa did not reverse the impaired acquisition and retention of the two-way active avoidance task in the MPTP-lesioned rats. These results also show that benserazide/l-dopa treatment by itself caused deficits in the acquisition and retention of this task. ANCOVA showed the following results of the interaction between treatments in the training session: MPTP lesion vs. day of the session interaction \( [F(1,43) = 3.70, p = 0.06]; \) benserazide/l-dopa treatment vs. day of the session interaction \( [F(1,43) = 0.68, p > 0.2]; \) MPTP lesion vs. benserazide/l-dopa treatment vs. day of the session interaction \( [F(1,43) = 2.26, p = 0.13] \). This absence of interaction between treatments and session day means that the failure of l-dopa therapy to improve retention scores of the MPTP-lesioned rats was not due to the lower avoidance training scores of these animals compared to the non-lesioned/vehicle group.

Discussion

The present data confirm and extend our previous results showing that the significant deficits presented by MPTP-lesioned rats in the two-way active avoidance tasks may be considered a model of the early learning and memory deficits observed in PD (Da Cunha et al., In Press). Several lines of evidence support this interpretation. The present results and previous experiments showed that the MPTP-lesioned animals did not present gross motor alterations and were not aphagic or adipsic (Da Cunha et al., In Press). In addition, a previous study performed in our laboratory showed that control and MPTP-lesioned animals presented the same reaction times to the sound and footshock during the two-way active avoidance task (Da Cunha et al., In Press). Therefore, these results suggest that the cognitive deficits observed in MPTP-lesioned rats are probably related to impairments in the memory acquisition and retention processes. These findings confirm other studies using different species, such as those by Schneider et al. (1988) who reported that monkeys exposed to low doses of MPTP also show cognitive impairment with mild or no motor deficits. Therefore, the picture observed in MPTP-lesioned animals is similar to the symptoms occurring in the early stage of PD when marked cognitive impairments can be observed while motor impairments are barely detected (Da Cunha et al., In Press; Dubois and Pillon, 1997). These clinical features begin to emerge when there is a 40–60% reduction of striatal DA (Obeso et al., 2000) and the neuron reduction is mainly restricted to the nigrostriatal DA neurons (Bonnet, 2000). In the present study, while the striatal level of DA decreased by approx. 40% in the MPTP-treated rats, no significant decrease in DA was observed in the frontal cortex, nucleus accumbens, hippocampus or amygdala, even though we also observed a relatively small damage in the VTA. This means that the MPTP lesion was really more specific for the midbrain DA neurons projecting to the striatum. Moreover, the observed pattern of striatal monoamine alterations showed that neither 5-HT nor NA neurons projecting to the striatum, nucleus accumbens, frontal cortex, or hippocampus were affected by this treatment. Harik et al. (1987) also showed that direct perinigral infusion of MPTP (at the same dose) in rats causes a lesion specific for the SNc and a more selective depletion of DA compared to other neurotoxins that deplete dopaminergic cells. However, as the decrease in the DA content observed in the frontal cortex was close to a statistically significant value, it is possible that some degree of frontal mesocortical DA denervation (probably caused in part by the minor VTA damage) affected the behaviour of the MPTP-lesioned rats. Here, it is important to mention that a
previous study by Albanese and Bentivoglio (1982) showed that some of the dopaminergic neurons in the rat SNc also project to the prefrontal cortex.

A prominent finding of this study was that the administration of the benserazide/L-dopa combination, at a dose sufficient to restore the striatal levels of DA, did not reverse the cognitive impairments induced in the MPTP-lesioned rats in the two-way active avoidance task, and also caused deficits in the acquisition and retention of this task by itself. It is noteworthy that the dose of L-dopa selected in the present study was effective in altering motor activity, as seen in the increased inter-trial crossings. Also, the effectiveness of the L-dopa dose is supported by the report of Menzaghi et al. (1997) showing that the same dose of L-dopa alters the turning behaviour of rats with unilateral MPTP-induced lesion of the SNc. However, in contrast to our findings, other studies indicated that DA replacement improved the performance of MPTP-treated monkeys in cognitive tasks (Fernandez-Ruiz et al., 1999; Schneider et al., 1998). This difference may be due to species differences or to different memory tasks used in the various studies. For example, another study observed the occurrence of ocular motor and visuospatial cognitive impairment in PD patients and in MPTP-lesioned monkeys, even after L-dopa therapy (Vila et al., 1996). In the present study the reduced baseline scores in the training session of the two-way active avoidance task caused by SNc lesion or L-dopa treatment would potentially confound the conclusion that L-dopa caused no absolute improvement of the memory retention scores of the test session. On this basis, this conclusion should be considered with some caution, until it is confirmed in future studies. Nevertheless, the absolute statistical analysis of the present data really favours this conclusion since in the three-way ANOVA taking the drug treatment and the MPTP-lesion as dependent variable and the training and test sessions as repeated measures no significant interaction was observed between MPTP-lesion and day of the session. This analysis favours the conclusion that the failure of L-dopa therapy to improve retention scores of the MPTP-lesioned rats was not due to the lower avoidance training scores of these animals compared to the non-lesioned/vehicle group. Furthermore, further analysis of the test session scores by two-way ANCOVA taking the drug treatment and the MPTP-lesion as dependent variables and the scores of the animals in the training session as covariate (not shown) showed a significant effect of the MPTP lesion and no effect of the L-dopa treatment independent of the baseline training avoidance scores, again favoring the conclusion that L-dopa did not improve the retention scores of the MPTP-lesioned rats despite their lower scores in the training session.

Besides the effectiveness of the benserazide/L-dopa treatment in restoring normal levels of striatal DA in the MPTP-treated rats, it should be noted that the treatment caused a marked increase in DA levels in the frontal cortex, hippocampus, amygdala and nucleus accumbens. Therefore, it is tempting to speculate that the failure of this drug combination to reverse the memory deficits in MPTP-treated rats is due to this overflow of DA into the extrastriatal brain structures. A strong support for this interpretation is provided by our data using control non-operated rats: the administration of benserazide/L-dopa to these rats caused a learning and memory impairment measured in the same active avoidance task. In this case, the cognitive impairment may result from the excessive DA levels in the other cerebral structures examined, most of them associated with the modulation of memory processes. Thus, the same dose necessary to restore the normal level of striatal DA is effective in improving motor performance and elevates the concentration of DA in other critical extrastriatal areas, thus acting within the memory impairing concentration range. This interpretation is in agreement with the study by Druzin et al. (2000) showing that the administration of a D2 DA receptor agonist into the rat prefrontal cortex impairs memory storage and executive working memory functions in a U-maze delayed task. These finding may be related to an inverse relationship between prefrontal and striatal DA systems initially proposed by Pycock et al. (1980) and Blanc et al. (1980), who observed that an increase in DA concentration in the prefrontal cortex causes a reduction in the striatal DA concentration. This finding was re-examined by Wilkinson (1997), who showed that it is widely supported in the literature concerning rodents, humans and non-human primates.

Although the above studies discourage the use of L-dopa therapy to treat PD cognitive symptoms, this does not imply that these cognitive symptoms are not related to the mesostriatal dopaminergic systems. Besides the observed impairment in the two-way active avoidance learning caused by the depletion of striatal DA in the MPTP-lesioned rats, other important findings suggest that mnemonic processes depend on a normal level of stimulation of the striatal DA receptors. For example, Packard and White (1991) and Packard and McGaugh (1994) showed improved cognitive performance after intra-striatal administration of a D2 receptor agonist to rats. Also, Schneider et al. (1994) observed a cognitive improving effect of the systemic administration of a D1 receptor agonist to MPTP-lesioned monkeys.

The present results may be related, at least in part, to the reported failure of L-dopa therapy to improve the cognitive impairments of PD patients reported by some authors (see above). Since the failure of this treatment
could be equally observed in some clinical studies and in this rat model of memory impairments related to PD, the data encourage the use of this model in future studies of alternative drug therapies for the treatment of cognitive impairments in PD. It will be interesting, for example, to use this model to test the effect of drugs acting on post-synaptic DA receptors which are mostly preserved after the SNc lesion. This and other studies using this rat model will lead not only to alternative putative treatments of the cognitive impairments of PD but also to the understanding of the role of basal ganglia in learning and memory processes.

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References


