Chapter 21
Distinct Effects of Intranigral L-DOPA Infusion in the MPTP Rat Model of Parkinson's Disease

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Abstract The potential neuroprotective or neurotoxic effects of 3,4-dihydroxyphenylalanine (L-DOPA) are yet to be understood. We examined the behavioral, immunohistochemical, tyrosine hydroxylase (TH) expression and neurochemical parameters after an intranigral administration of L-DOPA (10 μM) in rats.. L-DOPA elicited a 30.5% reduction in dopaminergic neurons, while 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) ($100 \mu g \mu L^{-1}$) produced a 53.6% reduction. A combined infusion of MPTP and L-DOPA generated a 42% reduction of nigral neurons. Motor parameters revealed that both the MPTP and L-DOPA groups presented impairments; however, the concomitant administration evoked a partial restorative effect. In addition, MPTP and L-DOPA separately induced reductions of TH protein expression within the substantia nigra. In contrast, the coadministration of MPTP and L-DOPA did not demonstrate such difference. The striatal levels of dopamine were reduced after MPTP or L-DOPA, with an increased turnover only for the MPTP group. In view of such results, it seems reasonable to suggest that L-DOPA could potentially produce dopaminergic neurotoxicity.

Keywords Animal model • L-DOPA • MPTP • Neurotoxicity • Parkinson's disease • Tyrosine hydroxylase

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Abbreviations

DA	Dopamine
L-DOPA	3,4-dihydroxyphenylalanine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD	Parkinson's disease
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
TH-ir	Tyrosine hydroxylase immunoreactive

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder the classical standard pharmacological protocol of which is polytherapy. The multitude of clinical approaches is detrimental to the overall comprehension of the physiological and molecular mechanisms that are affected by each drug individually. The possible neurotoxic consequence entailed by a given drug becomes difficult to trace as these effects are almost indistinguishable in clinical trials. Dopamine (DA) replacement by the administration of 3,4-dihydroxyphenylalanine (L-DOPA) is a gold standard drug for the symptomatic treatment of PD (Tedroff 1997). This therapy dramatically improves Parkinsonian symptoms. L-DOPA is converted by neuronal aromatic L-amino acid decarboxylase into DA, hence restoring DA levels in surviving neurons, but not halting neuronal death (Basma et al. 1995). Nevertheless, it is well known that chronic use of L-DOPA, especially in patients in advanced stages of the disease, leads to the development of motor complications that are very resistant to therapy, which aggravates disability in PD patients (Benbir et al. 2006). Yet another characteristic of L-DOPA administration is the occurrence of on–off motor variations, which is recognized by involuntary dyskinesia and psychiatric complications.

Animal models of PD have reported that acute L-DOPA treatment reversed the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced decrease in locomotion, rearing,

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and total activity (Sundstrom et al. 1990). For instance, 35 days of intraperitoneal L-DOPA treatment resulted in significant dyskinesia (Lundblad et al. 2004). Preclinical outcomes of in vitro models of neurodegeneration describe the neurotoxic effects of L-DOPA (Alexander et al. 1997; Ziv et al. 1997; Mytilineou et al. 2003), whereas in vivo trials in animal models provided controversial results (Muller et al. 2004). The variety of L-DOPA effects, in terms of its neurotoxic or neuroprotective potential and the mechanism through which DA is generated, exerts an effect despite the absence or reduction of DA innervation to target sites in the basal ganglia (Kostrzewa et al. 2005).

In this study, we hypothesized that an intranigral administration of L-DOPA 10 μM, which is a particular concentration that undergoes an important biotransformation within the substantia nigra (SN) (Sarre et al. 1998), could result in dopaminergic neurotoxicity, supporting the idea of a dual role of L-DOPA, and therefore, that of DA, in the nigrostriatal pathway. The aim of this study was to examine the effects of a single intranigral administration of L-DOPA, using as comparative parameter the experimental model of PD induced by the intranigral administration of MPTP in rats, which recapitulates several features of the disease (Lima et al. 2006, 2007; Reksidler et al. 2007, 2008). To obtain the needed data we verified the extent of dopaminergic neuronal loss inflicted by MPTP, L-DOPA, and their combination, named the MPTP+L-DOPA group, by estimating the number of tyrosine hydroxylase immunoreactive (TH-ir) neurons within the substantia nigra pars compacta (SNpc). Moreover, neurochemical analyses of the striatal content of DA and metabolites were conducted. Additionally, motor system function was examined under the open-field test. Complementarily, western blotting analyses were performed to verify the TH protein expression along the nigrostriatal pathway.

Material and Methods

Animals

Male Wistar rats (Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia-CEDEME facility of Universidade Federal de São Paulo-Escola Paulista de Medicina) weighing 280–320 g at the beginning of the experiments were used. Fifty rats were used in the behavioral tests, and the same animals were used for the immunohistochemistry analyses. An additional set of 45 rats was used for neurochemical and western blotting experiments. After the surgical procedure, the animals were returned to the same home cages where they were housed and maintained in a temperature-controlled room, located in our facility, (22 ± 2°C) on a 12 h light-dark cycle (lights on 7:00 a.m.) with free access to food and water. The animals used in this study were maintained and handled in accordance with the guidelines of the Ethical Committee of Universidade Federal do Paraná (UFPR) and Universidade Federal de São Paulo (UNIFESP), Brasil.

Experimental Protocol

On the first day of the experiment, the animals received bilateral guide cannula implants allowing for the introduction of an injection needle into the medial SNpc. After 7 days of recovery from the stereotaxic surgery, intranigral infusions of MPTP or L-DOPA were performed and another group received MPTP followed by L-DOPA in the same animals. Twenty-four hours after their infusion procedures, all the groups had their motor behavior recorded in the open-field. After assessment of their motor behavior, all the animals were intracardially perfused for brain fixation allowing for histological exam to determine the neuronal loss in the SNpc. Another set of animals was operated and underwent the same infusion procedure, but this set of animals was used for striatal neurochemical exam and TH protein expression study in the SN and striatum tissues.

Stereotaxic Surgery and Intranigral Injections

The animals were distributed at random, equally into the following groups: control (n = 19), sham (n = 19), MPTP (n = 19), L-DOPA (n = 19), and MPTP+L-DOPA (n = 19). Rats were anesthetized with diazepam (10 mg kg^{-1} i.p.) ketamine (90 mg kg^{-1} i.p.). All the groups, with the exception of the control, received a bilateral guide, cannula implanted 2.0 mm above the SNpc according to the following coordinates: anteroposterior (AP): −5.0 mm from bregma; mediolateral (ML): ±2.1 mm from midline; and dorsoventral (DV): −7.8 mm from skull, adapted from Paxinos and Watson (2005). All the operated rats received penicillin G-procaine (20,000 U in 0.1 ml, i.m.) after surgery.

After 7 days, the animals were manipulated and immobilized to perform the drug injections. A 30-gauge stainless injection needle was bilaterally introduced through the cannula for the administration of 1 μL of MPTP (100 μg μL^{-1}, prepared in sterile saline 0.9%, Sigma), 1 μL of L-DOPA (10 μM, prepared in sterile saline 0.9%, Sigma), which is a concentration ten fold greater than the cerebrospinal concentration of L-DOPA in human PD patients (Olanow et al. 1991), and 1 μL of MPTP (100 μg μL^{-1}) + 1 μL of L-DOPA

(10 μM). The control of the flow of the injection was made by using an electronic pump (Insight Instruments, Ribeirão Preto, Brazil) at a rate of 0.40 μL min^{-1} for 2.5 min, followed by 2 min with the needle in the injection site to avoid reflux. Sham operations followed the same procedure but were administered 1 μL of sterile saline 0.9%.

Behavioral Assessment

To determine behavioral alterations induced by intranigral administration of MPTP, L-DOPA, and their combination, we resorted to the open-field as a test of general activity. Fifty rats were distributed into five groups: nonoperated (n = 10), sham (n = 10), MPTP (n = 10), L-DOPA (n = 10), and MPTP+L-DOPA (n = 10). Each group, except the control and sham groups, received a bilateral administration of MPTP, L-DOPA, or both. The open-field was performed 24 h after the intranigral administrations to assess the maximal motor deficit elicited by MPTP (Lima et al. 2006).

We employed the open-field constructed according to Broadhurst (1960). The testing arena was round with a diameter of 97 cm. The circular wall was 32.5 cm high and was constructed of aluminum sheeting as was the arena floor. The walls were painted white. The arena floor was divided into three concentric circles. The inner circle had a diameter of 23 cm; the second circle had a diameter of 61 cm and the arena wall defined the outside circle. Each circle was divided into equal sized areas. The number of areas in the inner, middle, and outer circles was 1, 6, and 12 respectively. A 100-W ceiling light was situated 48 cm above the arena floor. Rats were observed individually for 5 min and the different groups were inter-mixed. Hand-operated counters were used to score the following parameters: latency time (time taken to initiate movement), locomotion frequency (number of floor units entered), rearing frequency (number of times the animals stood on their hind legs), and immobility time (number of seconds of lack of movement during testing). The apparatus was washed with a water–ethanol (5%) solution before behavioral testing to eliminate possible bias due to odors left by previous rats.

TH Immunohistochemistry

For the immunohistochemical study, the rats were deeply anesthetized with ketamine immediately after the behavioral test and were intracardially perfused with saline first, then with 4% of the fixative solution formaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed from the skulls and were immersed for 1 week in that fixative solution at 4ºC. Subsequently, the brains were placed in 30% sucrose solution for 48 h before sectioning. Four series of 30 μm thick sections were cut on a cryostat in the frontal plane and collected from the caudal diencephalon to the caudal midbrain. Tissue sections were incubated with primary antibody anti-TH, raised in rabbits, diluted in PBS containing 0.3% Triton X-100 (1:500; cat # AB152 Chemicon, CA, USA) overnight at 4°C. Biotin-conjugated secondary antibody incubation (1:200 cat # S-1000 Vector Laboratories, USA), was allowed for 2 h at room temperature. After several washes in PBS, antibody complex was localized using the ABC system (Vectastain ABC Elite kit cat # PK6101, Vector Laboratories, USA) followed by 3,3´-diaminobenzidine reaction with nickel enhancement. Slides were then dehydrated in ascending ethanol concentrations, cleared in xylene and coverslipped. An adjacent series was stained with cresyl violet to serve as a reference series for cytoarchitectural purposes.

To estimate the extent of neuronal loss within the midbrain due to MPTP, L-DOPA, and MPTP+L-DOPA, stereological methods were adopted. An operator performed blind counts, first assessing the dopaminergic cell population within the SNpc in control and sham animals. These were found to have preserved a normal cytoarchitectural appearance and TH immunostaining. Subsequently, the same investigator performed yet another blind count, assessing the same cell population from MPTP, L-DOPA, and MPTP+L-DOPA groups. All cell counts were done making use of the software Image-Pro Express 6. The selected areas were digitized through a digital camera DP71 (Olympus Optical Co, Japan) using an Olympus microscope BX50.

Neurochemical Determination of DA and Metabolites

Forty five rats were distributed into five groups: nonoperated (n = 6), sham (n = 6), MPTP (n = 6), L-DOPA (n = 6), and MPTP+L-DOPA (n = 6). The endogenous levels of DA and its nonconjugated metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by reverse-phase HPLC with electrochemical detection. Briefly, the system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 × 4.6 mm i.d., 4 μm particle size) fitted with a 4.0 × 3.0 mm precolumn (Security Guard Cartridges Fusion-RP). An electrochemical detector (ESA Coulochem Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV and a dual electrode analytical cell (ESA 5011A); a LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 μl loop. Oxidizing potentials were set

at 100 mV for the first electrode and at 450 mV for the second electrode. DA and metabolites were detected at the second electrode.

Each striatum was weighed in a measured volume (20% wt vol^{-1}) of 0.1 M perchloric acid and sodium metabisulfite 0.02% containing the internal standard 3,4-dihydroxybenzylamine at 100 ng mL^{-1}. The tissue samples were homogenized with an ultrasonic cell disrupter (Sonics). After centrifugation at 10,000 g for 30 min at 4°C, 20 μL of the supernatant was injected into the chromatograph. The mobile phase, used at a flow rate of 1 mL min^{-1}, had the following composition: 20 g citric acid monohydrated (Merck), 200 mg octane-1-sulfonic acid sodium salt (Merck), 40 mg ethylenediaminetetraacetic acid (EDTA) (Sigma), 900 mL HPLC-grade water. The pH of the buffer running solution was adjusted to 4.0 then filtered through a Nylon microfilter (pore size, 0.45 μm; Bioanalytical Systems, West Lafayette, IN). Methanol was added to give a final concentration of 10% (v/v). The concentrations of DA, DOPAC, and HVA were calculated using standard curves that were generated by determining in triplicate the ratios between three different known amounts of the internal standard. The unit was expressed as ng/g wet weight.

TH Protein Expression

To determine the expression profile of TH, we used 15 rats distributed into equivalent five groups mentioned previously: control (n = 3), sham (n = 3), MPTP (n = 3), L-DOPA (n = 3), and MPTP+L-DOPA (n = 3). Animals were killed by decapitation and their brains were quickly dissected. The SN and striatum were immediately frozen in dry ice and stored at −80°C until the lysis procedure was performed in a 1.5 mL Eppendorf tube by sonication in presence of an ice-cold buffer containing 50 mM Tris (pH 8.0), 250 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 0.25% sodium deoxycholate, 2 mM EDTA, 1 mM dithiothreitol (DTT), 20 μM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors (Complete tablet; Roche, Indianapolis, IN, USA).

After incubation on ice for 30 min, extracts were centrifuged at 12,000 × g for 40 min at 4° C, and the supernatants for protein extracts were collected and stored at −80°C for further western blotting analysis. The aliquot of supernatant was collected for total protein analysis (Lowry et al. 1951). Samples containing equal amounts of total protein (5 μg per lane) were boiled with SDS sample buffer and electrophoresed in 10% SDS-polyacrylamide gels in Mini Protean II Dual Slab Cell (Bio-Rad, USA). Proteins were electrophoretically transferred to PVDF membranes using a Mini transblot electrophoretic transfer cell (Bio-Rad, USA). Each membrane was blocked for 1 h in 10% nonfat dry milk/0.5% Tween-20 in Tris-buffered saline. Subsequently, each PVDF membrane was probed overnight at 4°C with mouse monoclonal antibodies against TH (1:5,000; cat # T2928 Sigma, USA) or β-tubulin (1:500; cat # MAB1637 Chemicon, USA) followed by several washes in TBST and incubation with an adequate secondary horseradish peroxidase-conjugated antibody (1:5,000; cat # 30021019 GE, USA) for 60 min, and visualized by chemiluminescence (cat # sc 2048 Santa Cruz Biotechnology, USA). The bands were quantified by using the software ImageJ 1.32j public domain (http://rsb.info.nih.gov.ij/).

Statistical Analysis

Differences in the number of cell counts and Western blotting data underwent analysis of variance (ANOVA) followed by Newman-Keuls test. The open-field data were concluded to be parametric by the Bartlet's test. ANOVA with repeated measurements was employed to detect differences among the treatments. For the behavioral and neurochemical data, the Tukey test was used as post hoc when indicated by ANOVA. Differences were considered significant if $P < 0.05$. The values were expressed as mean ± S.E.M.

Results

Behavioral Assessment

The data obtained for latency in initiating movement (Fig. 1a) revealed a significant increase in this parameter in the groups administered with L-DOPA ($P < 0.05$), MPTP ($P < 0.001$), and MPTP+L-DOPA ($P < 0.05$) in comparison with the control group [$F(4.32) = 14.57$; $P < 0.05$]. The MPTP group exhibited a more pronounced increase in this parameter in comparison with the L-DOPA ($P < 0.05$) and MPTP+L-DOPA ($P < 0.01$) groups. Indeed, the immobility time presented similar alterations.

The analyses of ambulatory behavior were determined by the locomotion frequency depicted in Fig. 1b. That figure shows a significant reduction in the groups that received L-DOPA ($P < 0.05$), MPTP ($P < 0.001$), and their combination MPTP+L-DOPA ($P < 0.05$) in comparison with the control group [$F(4,32) = 14.19$; $P < 0.05$]. As expected, the animal model of PD induced by the MPTP group presented a significant reduction in the locomotion frequency in comparison with the L-DOPA ($P < 0.05$) and MPTP+L-DOPA ($P < 0.01$) groups. Rearing frequencies (Fig. 1c) indicated

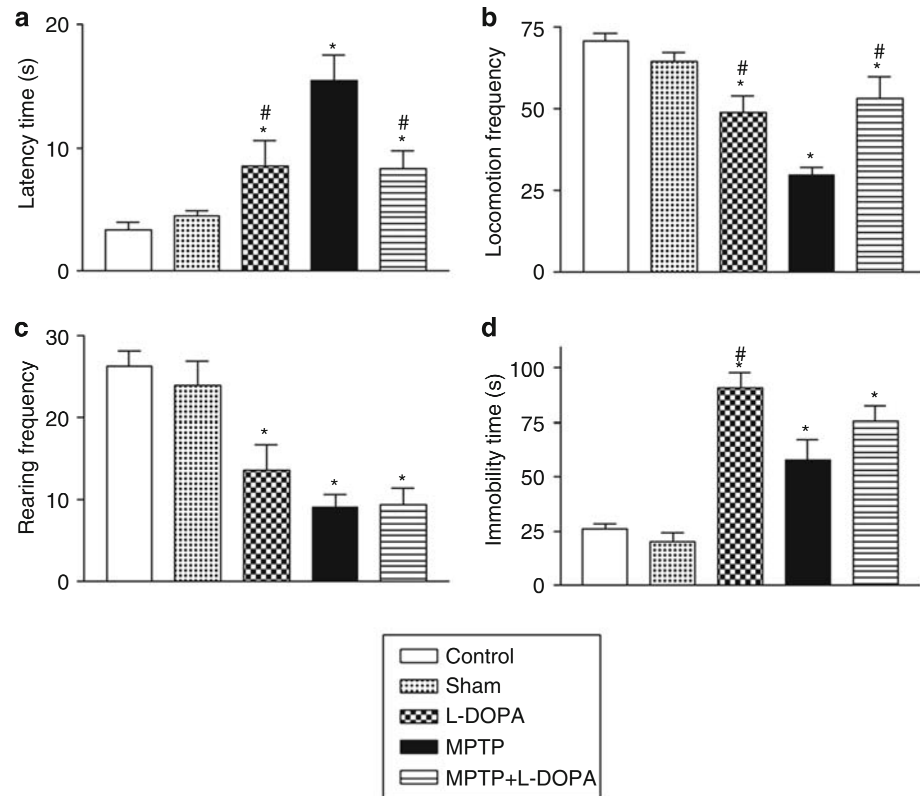

Fig. 1 Effect of intranigral infusions of MPTP, L-DOPA or MPTP+L-DOPA in the open-field test. (**a**) Latency time, (**b**) locomotion frequency, (**c**) rearing frequency, (**d**) immobility time. The values are expressed as mean ± S.E.M. ($n=10$ animals per group). *P<0.05 compared to control group; #P<0.05 compared to MPTP group. ANOVA followed by the Tukey test

that L-DOPA (P<0.01), MPTP (P<0.001), and MPTP+L-DOPA (P<0.001) showed a significant decrease in this exploratory parameter when the animals were compared with the control group [F(4,32)=11.64; P<0.01].

Figure 1d depicts a significant increase in this parameter in the following groups: L-DOPA (P<0.001), MPTP (P<0.01), and MPTP+L-DOPA (P<0.001) in comparison with the control [F(4,32)=31.79; P<0.01]. Moreover, the L-DOPA group presented a significant increase in this motor sign when compared with the MPTP (P<0.01) group.

TH Immunohistochemistry

The effects of L-DOPA, MPTP, and MPTP+L-DOPA on the number of TH-ir neurons were examined by immunohistochemistry. As depicted in Figs. 2 and 3, the intranigral administration of MPTP, L-DOPA, and MPTP+L-DOPA caused a similar loss of TH-ir neurons within the SNpc [F(3,30)=55.93; P<0.001] in comparison with the control group. MPTP administration provoked the more pronounced loss (53.6%) of TH-ir neurons in the SNpc (P<0.001) in comparison with the control group. Otherwise, the L-DOPA group presented a 30.5% (P<0.001) reduction in the number of TH-ir neurons within the SNpc in comparison with the control group. Conversely, the number of TH-ir neurons from the L-DOPA group was significantly increased (23.2%; P<0.001) in comparison with the MPTP group. The combination of MPTP and L-DOPA also resulted in a significant loss of 42% (P<0.001) of the TH-ir neurons within the SNpc, compared with the control group.

Neurochemical Determination of DA and Metabolites

Figure 4a, illustrates that levels of DA were significantly lower in the striatum of L-DOPA (P<0.001), MPTP (P<0.001), and L-DOPA+MPTP (P<0.001) groups in comparison with sham and control groups [F(4,24)=5.45; P<0.001]. This figure also demonstrates that DOPAC levels were not altered for the groups treated with L-DOPA when compared with control and sham groups (Fig. 4b). However, the MPTP group revealed a significant increase in the DOPAC level in comparison with the control (P<0.05), sham (P<0.05), and L-DOPA (P<0.05) groups [F(4,28)=3.91; P<0.01] (Fig. 4b). Similarly, HVA showed an increase solely in the striatum of the MPTP group, when compared with the control (P<0.05), sham (P<0.01), L-DOPA (P<0.01), and MPTP+L-DOPA (P<0.01) groups [F(4,32)=5.47; P<0.002] (Fig. 4c).

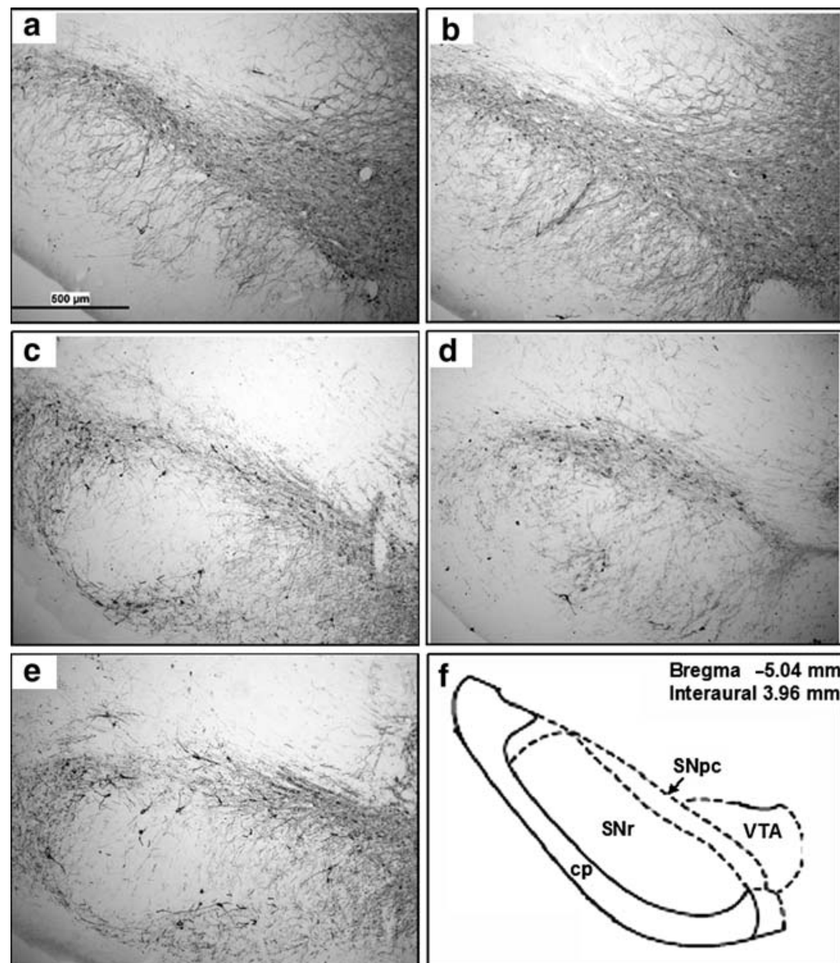

Fig. 2 Photomicrograph of representative sections of tyrosine hydroxylase immunoreactive neurons (TH-ir) in the substantia nigra of the following groups: (**a**) Control, (**b**) Sham, (**c**) MPTP, (**d**) L-DOPA, (**e**) MPTP+L-DOPA. Panel **f** is a schematic drawing from the area previously depicted (Paxinos and Watson 2005). Legend *cp* cerebral peduncle basal, *SNpc* substantia nigra pars compacta, *SNr* substantia nigra reticulate, *VTA* ventral tegmental area, scale bar 500μm

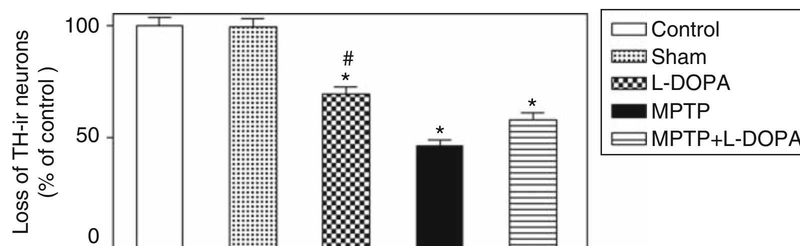

Fig. 3 Effects of intranigral infusions of MPTP, L-DOPA or MPTP+L-DOPA on the percentage of loss of tyrosine hydroxylase immunoreactive neurons (TH-ir) in the substantia nigra pars compacta SNpc of rats. The values are expressed as mean ± S.E.M. compared to the control group ($n=10$ animals per group). *P <0.05 compared to the control group; $^{\#}$P<0.05 compared to MPTP group; ANOVA followed by the Newman–Keuls test

TH Protein Expression

Western blot analysis of the SN and the striatum revealed a single band of molecular mass of 60 kDa in all groups. Densitometric analyses showed that intranigral administrations of MPTP and L-DOPA produced, in their respective groups, a significant decrease of TH expression in the SN [$F(4,45)=4.05$; $P<0.05$] in comparison with the controls (Fig. 5a). The group lesioned with MPTP presented a significant reduction of about 30% ($P<0.05$) in the TH expression within the SN. Similarly, the L-DOPA group presented a reduction of 28% ($P<0.05$) in the expression of this protein in the SN. The analysis of the SN obtained after MPTP+L-DOPA administration revealed an absence of

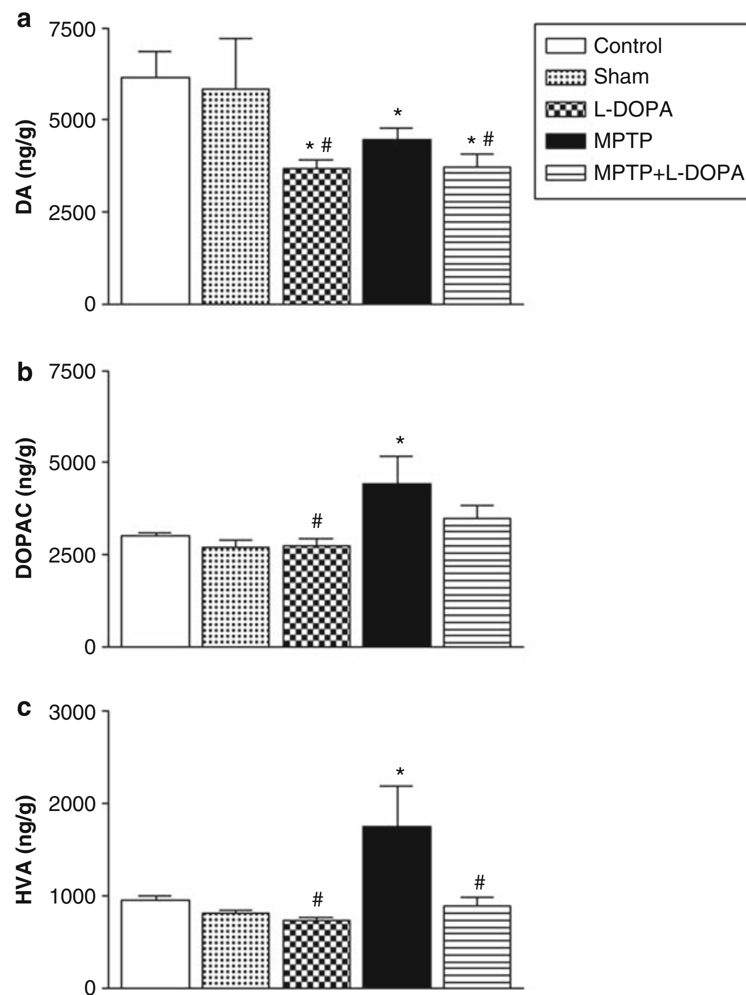

Fig. 4 Neurochemical determination of DA and metabolites in the striatum. Panel (**a**) depicts the levels of DA, panel (**b**) levels of DOPAC and panel (**c**) levels of HVA. The values are expressed as mean ± S.E.M. (n=8 animals per group). *P<0.05 compared to control group; #P<0.05 compared to MPTP group. ANOVA followed by the Tukey test

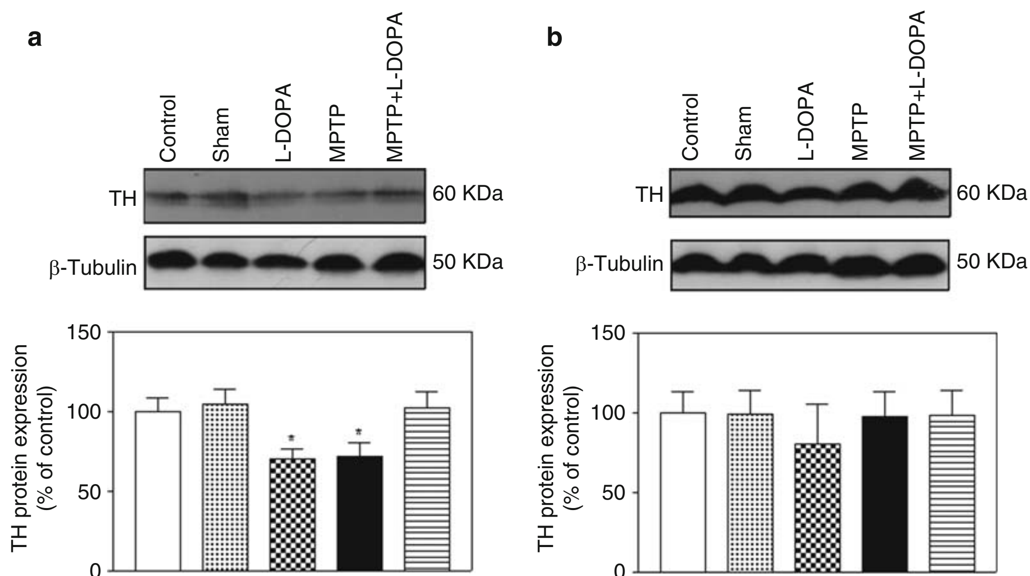

Fig. 5 Western blot analyses of TH expression in the substantia nigra (**a**) and striatum (**b**) of groups which received intranigral infusions of MPTP, L-DOPA or MPTP+L-DOPA. A representative immunoblot is shown. The values are expressed as mean ± S.E.M. for three animals in each group. *P<0.05 compared to the control group. ANOVA followed by the Newman–Keuls test

statistical difference in TH expression in comparison with the control group. The analyses of the striatum tissue did not exhibit significant variations in the TH protein expression among the groups included in this study (Fig. 5b).

Discussion

Our experiments demonstrated that a single intranigral administration of L-DOPA induced nigrostriatal dopaminergic destruction similar to that inflicted by MPTP. Analyses of TH protein expression revealed that L-DOPA caused a reduction in the expression of this protein, one very similar to that caused by MPTP in the SN. In parallel, the content of DA was reduced in the striatum after MPTP or L-DOPA, but an increased DA turnover was detected only for the MPTP group. Of note, the combined administration of MPTP and L-DOPA resulted in a relevant reduction of neurotoxicity in TH expression within the SN than that inflicted by MPTP and L-DOPA alone, as demonstrated in our TH expression experiments.

L-DOPA is the most commonly prescribed drug for the symptomatic treatment of PD. Despite this, L-DOPA per se presents a serious limitation due to its pharmacological mechanisms, which depend upon the integrity of a minimal neuronal population capable of converting this precursor into DA. Unfortunately, L-DOPA and other anti-Parkinsonian drugs are ineffective in controlling ongoing cell death, and more recently, the notion that L-DOPA can produce dopaminergic neurotoxicity has gained strength (Bendir et al. 2006). Sarre et al. (1998) confirmed that there is an important biotransformation of L-DOPA into DA within the SN of rats infused with L-DOPA directly into this structure. Consequently, it has been postulated that high DA concentrations formed by L-DOPA may cause side effects in PD patients. There are reports stating that approximately 50% of the patients develop motor complications 5 years after the initiation of L-DOPA therapy. This figure increases to approximately 70% after 15 years (Miyawaki et al. 1997). The incidence of such motor problems reaches almost 100% in patients with early onset of PD (Quinn et al. 1987; Golbe 1992; Schrag and Quinn 2000; Thobois et al. 2005).

Our behavioral results partially corroborate these previous data, which denote that L-DOPA alone produced some level of similarity with MPTP, in terms of impairment in the motor parameters. The analysis of latency time revealed that L-DOPA caused a smaller increase in this time, compared with MPTP. This augmented latency time can be related to the manifestation of bradykinesia that occurs in PD. Similarly, the MPTP+L-DOPA group presented approximately the same profile of latency time as the L-DOPA group. In this sense it is possible to suggest that intranigral L-DOPA treatment, right after MPTP lesion, resulted in distinct modulatory locomotion patterns in the MPTP+L-DOPA and L-DOPA groups. An analogous situation of responses was observed in relation to locomotion frequency parameter. The L-DOPA, MPTP, and MPTP+L-DOPA groups showed significant reductions of locomotion frequencies in comparison with the control group. Although the hypokinesia observed in the two groups treated with L-DOPA is a characteristic feature of a PD model, a significant increase in locomotion frequency in the L-DOPA groups was found in comparison with the MPTP group. Such a result reinforces the notion that patterns of dopaminergic modulation elicited by L-DOPA may exist. Additionally, rearing frequency was reduced in the experimental groups in comparison with controls. Such observations demonstrate the property of MPTP and L-DOPA in the induction of dopaminergic impairment according to the experimental protocol adopted. Immobility time was the only behavioral parameter that L-DOPA alone produced, an effect similar to that of MPTP in the impairment of motor function. The evidence, so far, suggests the possible existence of different patterns of modulatory effects in the dopaminergic nigrostriatal pathway inflicted by L-DOPA.

Several studies have demonstrated that the intranigral administration of MPTP is an established model of the early phase of human PD (Da Cunha et al. 2001, 2002; Miyoshi et al. 2002; Ferro et al. 2005; Braga et al. 2005; Perry et al. 2005; von Bohlen und Halbach 2005; Lima et al. 2006, 2007; Reksidler et al. 2007, 2008). At this point, a note of caution should be added: we evaluated the effects produced by L-DOPA, on dopaminergic neurons, using the MPTP model of PD as a standard of comparison. In this sense, we decided to adopt the same protocol of administration (intranigral) for both substances (MPTP and L-DOPA). Moreover, such approach of neurodegeneration in rats is usually performed with a single intranigral administration of MPTP producing massive effects 24 h after its intranigral infusion (Lima et al. 2006, 2007; Reksidler et al. 2007). Otherwise, a previous study demonstrated that repeated intranigral MPTP administrations did not generate progressive neuronal death, indicating that a single MPTP administration produces a very similar pattern of alterations (Reksidler et al. 2008).

Similar to the MPTP-induced lesion, L-DOPA produced diminishment in the percentage of the TH-ir neurons in comparison with controls, smaller still in comparison with the MPTP group. Despite its demonstrated potential to cause dopaminergic neurotoxicity when injected in the SNpc, L-DOPA produced a mild reduction of TH-ir neurons when compared with MPTP. The combined administration of MPTP and L-DOPA generated a similar dopaminergic

neurodegeneration inflicted by MPTP alone. In this context, we did not observe any immediate property of L-DOPA in the rescue of dopaminergic neurons from the MPTP insult.

Western blotting data showed that the striatal expression of TH protein was not altered in the groups tested. This response is consistent to Maeda and colleagues (1997), who reported no alteration in the relative density of striatal TH immunolabeling in the MPTP+L-DOPA group compared with the MPTP-saline group. In contrast, a significant reduction of TH expression in both L-DOPA and MPTP groups in the SN was verified when compared with controls. These data are suggestive of damage in the nigrostriatal pathway. Surprisingly, the MPTP+L-DOPA group did not exhibit a reduction of TH expression in the SN. That intriguing fact makes for compelling evidence of neuronal plasticity unchained by L-DOPA subsequent to the severe dopaminergic damage inflicted by MPTP. These data are in stark contrast to that encountered by Myers et al. (1999), which described that L-DOPA appears to enhance neuronal degeneration in animals the dopaminergic function of which has been compromised by neurotoxin assault.

The metabolism of L-DOPA and DA produces quinones, semiquinones, hydrogen peroxide, and other oxyradicals, which are believed to be toxic to the SN neurons (Fahn and Cohen 1992). Exogenous L-DOPA administration significantly decreased the DA levels within the striatum, corroborating previous data showing that high doses of L-DOPA are not accompanied by significant increases in extracellular DA; therefore, the observed effects would be due to the direct stimulation of DA receptors by the L-DOPA itself (Fisher et al. 2000). Such inference could be correct, because concentrations of the DA metabolites DOPAC and HVA were not found to be increased in the striatum.

The present results support earlier studies (Gross et al. 2003; Guigoni et al. 2005; Bendir et al. 2006; Konitsiotis and Tsironis 2006) indicating that L-DOPA alone is capable of inducing impairment in the dopaminergic system. In contrast, our results clearly demonstrated that the intranigral administration of L-DOPA, after previous MPTP infusion, presented a strong compensatory effect circumscribing motor behavior and TH expression parameters. Nevertheless, our neurochemical findings suggested that effect probably did not occur due to a consequence of L-DOPA, which provided additional supplies for DA synthesis in the neurons that survived from the MPTP lesion. Therefore, L-DOPA did not increase the striatal DA levels or DA metabolites, according to the adopted protocol. An alternative explanation could suggest a direct activation of dopaminergic receptors by L-DOPA, resulting in the compensatory effect. However, animals that received a single intranigral L-DOPA administration manifested a typical histological, behavioral, and neurochemical alteration of lesion of the nigrostriatal pathway.

It has been suggested that unlike the effects of L-DOPA in the normal SN, the drug may be toxic to the partially damaged SN neurons in PD patients (Rajput 2001). Nevertheless, we suggest that L-DOPA is more associated with a compensatory or even neuroprotective action when administered after a lesion. Thus, corroborating the work of Gross and colleagues (2003), we propose that L-DOPA may exert distinct effects on the dopaminergic system, mainly depending on the integrity of the nigrostriatal pathway. Future studies are warranted to complement the understanding of the interaction of L-DOPA therapy with the dopaminergic system in normal and in PD scenarios.

Conflicts of interest statement we declare that we have no conflict of interest.

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References

Alexander T, Sortwell CE, Sladek CD et al (1997) Comparison of neurotoxicity following repeated administration of *l*-dopa, *d*-dopa, and dopamine to embryonic mesenphalic dopamine neurons in cultures derived from fisher 344 and Sprague-Dawley donors. Cell Transplantation 6:309–315

Basma RB, Morris EJ, Niklas WJ et al (1995) L-DOPA cytotoxicity to PC12 cells in culture is via its autooxidation. J Neurochem 64: 825–832

Benbir G, Ozekmekci S, Cinar M et al (2006) Features associated with the development of hallucinations in Parkinson's disease. Acta Neurol Scand 114:239–243

Bendir G, Özekmekçi S, Apaydin H et al (2006) A hospital-based study: risk factors in development of motor complications in 555 Parkinson's patients on levodopa therapy. Clin Neurol Neurosurg 108:726–732

Braga R, Kouzmine I, Canteras NS et al (2005) Lesion of the substantia nigra pars compacta impairs delayed alternation in a Y-maze in rats. Exp Neurol 192:134–141

Broadhurst PL (1960) Experiments in psychogenetics. In: Eisenk HJ (ed) Experiments in personality. Routledge and Kegan Paul, London

Da Cunha C, Gevaerd MS, Vital MABF et al (2001) Memory disruption in rats with nigral lesions induced by MPTP: a model for early Parkinson's disease amnesia. Behav Brain Res 124:9–18

Da Cunha C, Angellucci MEM, Canteras NS et al (2002) The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson´s disease memory disabilities. Cell Mol Neurobiol 22:227–237

Fahn S, Cohen G (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 32:804–812

Ferro MM, Bellíssimo MI, Anselmo-Franci JA et al (2005) Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of early phase of Parkinson's disease: Histological, neurochemical, motor and memory alterations. J Neurosci Methods 148:78–87

Fisher A, Biggs CS, Eradiri O et al (2000) Dual effects of L-3, 4-dihydroxyphenylalanine on aromatic L-amino acid decarboxilase,

dopamine release on motor stimulation in the reserpine-treated rat: evidence that behavior is dopamine independent. Neuroscience 95:97–111

Golbe LI (1992) Young-onset Parkinson's disease: a clinical review. Neurology 41:168–173

Gross CE, Ravenscroft P, Dovero S et al (2003) Pattern of levodopa-induced striatal changes is different in normal and MPTP-lesioned mice. J Neurochem 84:1246–1255

Guigoni C, Dovero S, Aubert I et al (2005) Levodopa-induced dyskinesia in MPTP-treated macaques is not dependent on the extend and pattern of nigrostriatal lesioning. Eur J Neurosci 22:283–287

Konitsiotis S, Tsironis C (2006) Levodopa-induced dyskinesia and rotational behavior in hemiparkinsonian rats: independent features or components of the same phenomenon? Behav Brain Res 170:337–341

Kostrzewa RM, Nowak P, Kostrzewa JP et al (2005) Peculiarities of L-DOPA treatment of Parkinson's disease. Amino Acids 28:157–164

Lima MMS, Reksidler AB, Zanata SM et al (2006) Different parkinsonism models produce a time dependent induction of COX-2 in the substantia nigra of rats. Brain Res 1101:117–125

Lima MMS, Andersen ML, Reksidler AB et al (2007) The role of the substantia nigra pars compacta in regulating sleep patterns in rats. PLoS ONE 2:e513

Lowry OH, Rosebrough NJ, Farr AL et al (1951) Protein measurement with the Folin protein reagent. J Bio Chem 193:265–275

Lundblad M, Picconi B, Lindgreen H et al (2004) A model of L-dopa-induced dyskinesia in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 16:110–123

Maeda T, Cheng N-N, Kume T et al (1997) L-dopa neurotoxicity is mediated by glutamate release in cultured striatal neurons. Brain Res 771:159–162

Miyawaki E, Lyons K, Pahwa R et al (1997) Motor complications of chronic levodopa therapy in Parkinson's disease. Clin Neuropharmacol 20:523–530

Miyoshi E, Wietzikoski S, Camplessei M et al (2002) Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. Brain Res Bull 58:41–47

Muller T, Hefter H, Hueber R et al (2004) Is levodopa toxic? J Neurol 251:44–46

Myers CS, Halladay AK, Widmer DA et al (1999) Neurotoxic effects of amphetamine plus L-DOPA. Prog Neuro-Psychopharmacol Biol Psychiatry 23:731–740

Mytilineou C, Walker RH, Jnobaptiste R et al (2003) Levodopa is toxic to dopamine neurons in an in vitro but not an in vivo model of oxidative stress. J Pharmacol Exp Ther 304:792–800

Olanow CW, Gauger BA, Cedarbaum MJ (1991) Temporal relantionships between plasma and cerebrospinal fluid pharmacokinetics of levodopa and clinical effect in Parkinson´s disease. Ann Neurol 29:556–569

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates, 5th edn. Academic, San Diego, CA

Perry JC, Hipólide DC, Tufik S et al (2005) Intra-nigral MPTP lesion in rats: behavioral and autoradiography studies. Exp Neurol 195:322–329

Quinn N, Critchley P, Marsden CD (1987) Young-onset Parkinson's disease. Mov Disord 2:73–91

Rajput AH (2001) Levodopa prolongs life expectancy and is non-toxic to substantia nigra. Parkinsonism Relat Disord 8:95–100

Reksidler AB, Lima MMS, Zanata SM et al (2007) The COX-2 inhibitor parecoxib produces neuroprotective effects in MPTP-lesioned rats. Eur J Pharmacol 560(2):163–175

Reksidler AB, Lima MMS, Dombrowski P et al (2008) Repeated intranigral MPTP administration: a new protocol of prolonged locomotor impairment mimicking Parkinson's disease. J Neurosci Methods 167:268–277

Sarre S, Vandeneed D, Ebinger G et al (1998) Biotransformation of L-DOPA to dopamine in the substantia nigra of freely moving rats: effect of dopamine receptor agonists and antagonists. J Neurochem 70:1730–1739

Schrag A, Quinn N (2000) Dyskinesias and motor fluctuations in Parkinson's disease: a community-based study. Brain 123: 2297–2305

Sundstrom E, Fredriksson A, Archer T (1990) Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: a model for Parkinson´s disease. Brain Res 528:181–188

Tedroff JM (1997) The neuroregulatory properties of L-DOPA. A review of the evidence and potential role in the treatment of Parkinson´s disease. Rev Neurosci 8:195–204

Thobois S, Delamarre-Damier F, Derkinderen P (2005) Treatment of motor dysfunctions in Parkinson's disease: an overview. Clin Neurol Neurosurg 107:269–281

von Bohlen und Halbach O (2005) Animal models of Parkinson´s disease. Neurodegener Dis 2:313–320

Ziv I, Zilkha-Falb R, Offen D et al (1997) Levodopa induces apoptosis in cultured neuronal cells-a possible accelerator of nigrostriatal degeneration in Parkinson´s disease? Mov Disord 12:17–23