Current Trends in Neurology
Vol. 7, 2013

Original Communication

Antidepressant-like effect of curcumin in 6-hydroxydopamine model of Parkinson's disease

Raisa Wendhausen Gradowski, Ronise M. Santiago, Tiago Zaminelli, Taysa Bervian Bassani, Janaína Kohl Barbiero, Suelen L. Boschen, Roberto Andreatini and Maria A. B. F. Vital*
Pharmacology Department, Federal University of Paraná, Brazil

ABSTRACT

One of the most common nonmotor symptoms of Parkinson's disease (PD) is depression, which affects approximately 35% of patients and may significantly impair their quality of life. Curcumin, a major active compound of turmeric (*Curcuma longa*), has been found to have several pharmacological properties. It has anti-inflammatory activity, inhibits monoamine oxidase (MAO), and has neuroprotective effects. This compound has already been extensively studied in various models of depression and, more recently, it has been studied in PD models. However, little is known about its effects on depression associated with PD. The present study investigated the antidepressant-like effect of curcumin in an animal model of 6-hydroxydopamine (6-OHDA)-induced PD. Male Wistar rats received a bilateral intranigral infusion of 6-OHDA, and the sham group received the vehicle. All of the rats were treated for 21 days with 30 mg/kg curcumin orally (p.o.) or vehicle (sunflower oil, p.o.) starting 1 h after surgery. The animals were subjected to the forced swim test and the sucrose preference test. Curcumin exerted an antidepressant-like effect in both tests. Neurochemical analyses were then performed. Curcumin increased the levels of dopamine and its metabolites in the striatum. Immunohistochemical analysis showed that curcumin was able to prevent the death of dopaminergic neurons in the substantia nigra *pars compacta* (SNpc). These findings suggest antidepressant-like and neuroprotective effects of curcumin in the animal model of 6-OHDA-induced PD.

KEYWORDS: Parkinson's disease, curcumin, depression, 6-hydroxydopamine

ABBREVIATIONS

5-HIAA	-	5-hydroxyindoleacetic acid
5-HT	-	Serotonin (5-hydroxytrypatamine)
6-OHDA	-	6-hydroxydopamine
DA	-	Dopamine
DHPG	-	Dihydroxyphenylglycol
DOPAC	-	3,4-dihydroxyphenylacetic acid
HPLC	-	High-performance liquid chromatography
HVA	-	Homovanillic acid
MAO	-	Monoamine oxidase
NE	-	Norepinephrine
PBS	-	Phosphate-buffered saline
PD	-	Parkinson's disease
SNpc	-	Substantia nigra *pars compacta*
SNr	-	Substantia nigra *pars reticulata*
TH	-	Tyrosine hydroxylase

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra *pars compacta* (SNpc) [1-3].

*Corresponding author:
Maria Aparecida Barbato Frazão Vital,
Universidade Federal do Paraná, Setor de Ciências Biológicas, Departamento de Farmacologia,
Av. Francisco H. dos Santos s/n, Curitiba,
Paraná, 81531-990, Brasil.
vital@ufpr.br

PD patients exhibit a large number of motor impairments associated with the disease, such as bradykinesia, tremor at rest, postural instability and freezing [2]. However, these patients may also develop nonmotor symptoms, including olfactory problems, constipation, sleep disorders, anxiety, dementia and depression [4]. Depression associated with PD occurs in approximately 35% of PD cases. Depression in PD is related to psychosocial stress caused by the disease and the neurodegeneration process [5, 6]. This neurodegeneration in PD is mainly associated with dopaminergic neurons, but serotoninergic, noradrenergic and cholinergic systems are also involved [6, 7]. The precise etiology of depression in PD has not been elucidated, but numerous pathways have been linked to this process. In major depression, monoaminergic dysfunction, neuroinflammation, and neurodegeneration play important roles in the disease pathophysiology [8, 9]. Depression associated with PD appears to be associated with an increase in inflammation pathways, in which several inflammatory mediators are upregulated [6]. This increase could result from an uncontrolled process that may contribute to neuronal death [10]. Despite the importance of serotonin (5-hydroxytrypatamine [5-HT]) in major depression, the role of this neurotransmitter in PD-related depression has not been elucidated [6]. Studies have been inconclusive with regard to treating PD-related depression with conventional antidepressant drugs that inhibit 5-HT reuptake. The depletion of tryptophan (i.e., the 5-HT precursor) did not promote depressive symptoms in PD patients without depression, suggesting that other monoamines could be more involved in the emergence of this PD symptom [6, 11, 12]. Parkinson's disease patients present impairments in dopaminergic pathways related to reward and mood systems. A reduction of dopamine levels in frontal and subcortical regions has been observed in PD patients with depression, thus linking this neurotransmitter with PD-related depression [6]. Additionally, norepinephrine (NE) appears to play an important role in the emergence of PD-related depression. Depressive PD patients presented greater neurodegeneration in the locus coeruleus, a structure linked to noradrenergic function, compared with non-depressive PD patients [6].

Curcumin is a major active compound of *Curcuma longa* L. It is a molecule with multiple pharmacological properties, such as antioxidant, anti-inflammatory and neuroprotective effects [13, 14]. Based on behavioral and biochemical analyses in depression models, curcumin has been shown to exert an antidepressant-like effect. Curcumin enhanced monoamine levels, promoted hippocampal neurogenesis by increasing brain-derived neurotrophic factor levels, and inhibited monoamine oxidase (MAO), an enzyme linked to monoamine metabolism [15-17]. Most studies that have used PD models have been performed *in vitro*. The neuroprotective effects of curcumin have been observed against toxins, such as MPTP and 6-OHDA [18, 19]. *In vitro* studies have shown that curcumin is able to bind α-synuclein to prevent its aggregation and consequently Lewy body formation [20, 21]. *In vivo* studies also have shown that pretreatment with curcumin protects against dopaminergic neuron death caused by toxin administration in the SNpc [22]. Studies in which curcumin treatment was begun immediately after toxin exposure found that it inhibited MAO-B in the striatum [19]. Curcumin was also shown to prevent dopaminergic neuron death by inhibiting the glial activation response in the striatum and SNpc [23].

To date, little is known about the effects of curcumin in PD-related depression. Given the multiple mechanisms of action of curcumin and the multifactorial pathways of depression in PD [14], we investigated whether curcumin would be effective in an animal model of PD-related depression. We injected 6-OHDA bilaterally into the SNpc and evaluated whether curcumin could protect against the depressive-like effect induced by the neurotoxin. To support the behavioral studies, neurochemical and immunohistochemical analyses were also performed.

MATERIALS AND METHODS

Animals

Male Wistar rats, 290-320 g, were used. They were randomly housed under standard conditions of temperature (22 ± 2 ºC) and illumination (12 h/12 h light/dark cycle). They had free access to water and food throughout the experiment.

The studies were performed in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. The protocol complied with the recommendations of the Federal University of Paraná and was approved by the Institutional Ethics Committee (protocol no. 590).

Experimental design

The animals were randomly distributed into the following four groups (*n* = 9-14/group): sham-vehicle, sham-curcumin, 6-OHDA-vehicle and 6-OHDA-curcumin. All of the rats underwent stereotaxic surgery. The treatment began one hour after surgery when the rats recovered from anesthesia, ending 21 days later. The animals orally (p.o.) received 30 mg/kg curcumin (Sigma, St. Louis, MO, USA) or vehicle (sunflower oil) daily between 9:00 AM and 10:00 AM. The curcumin dose was based on a pilot study performed in our laboratory (unpublished data). Three different concentrations of curcumin (10, 20, and 30 mg/kg, p.o.) were tested in an acute administration protocol. After three administrations (24, 8 and 1 h before the test), the rats were subjected to the forced swim test. Although all three curcumin doses exerted antidepressant-like effects, the 30mg/kg dose of curcumin showed the most significant effect and the effect was similar to that of imipramine treatment.

The study was divided into two parts (Fig. 1). In both experiments, the animals received the same treatment schedule as described above. In Experiment 1 (*n* = 10-12/group), all of the rats were subjected to the open field test 24 h and 21 days after stereotaxic surgery. On day 21, the training session of the forced swim test was conducted. Twenty-four hours later, the test session of the forced swim test was performed.

In Experiment 2 *(n* = 12-14/group), all of the rats were subjected to the sucrose preference test prior to surgery to determine the basal values of sucrose preference. Rats with sucrose preference < 75% were discarded from the study. Rats with sucrose preference > 75% underwent stereotaxic surgery. The sucrose preference test was performed 7, 14, and 21 days after surgery. After the sucrose preference test, some of the rats (*n* = 9-10/group) were immediately decapitated, and the striatum and hippocampus were dissected. The remaining animals (*n* = 3-4/group) were submitted to a perfusion process for immunohistochemistry.

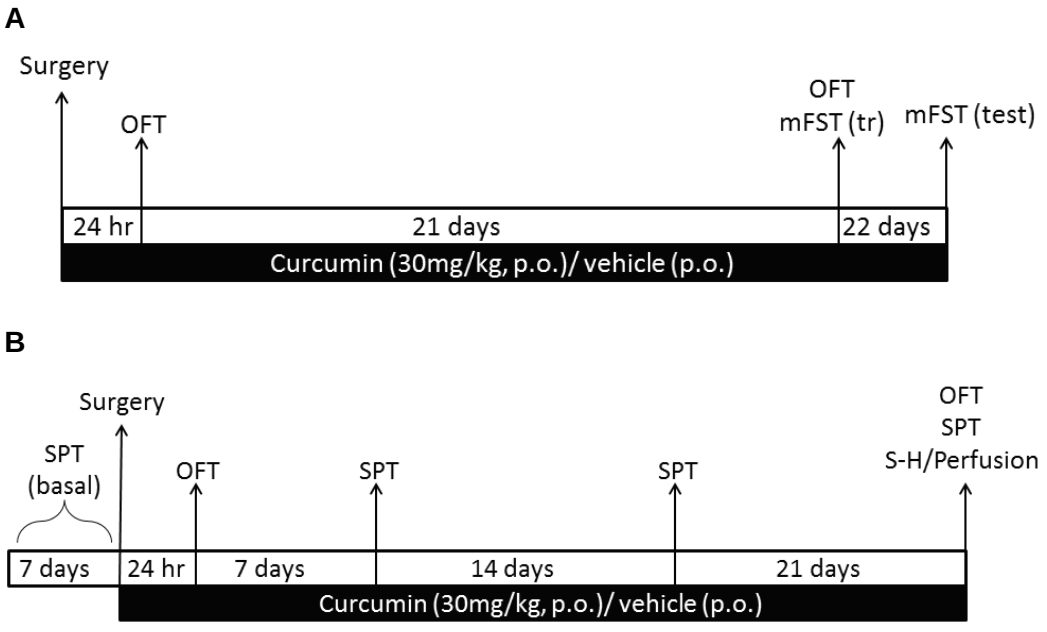

Fig. 1. Experimental design. Experiment 1 (A) and experiment 2 (B). OFT – open field test; mFST(tr) – modified forced swim test training session; mFST(test) – modified forced swim test session; SPT – sucrose preference test; S-H – dissection of *striatum* and *hippocampus*.

Stereotaxic surgery

6-OHDA was infused into the SNpc during stereotaxic surgery. All of the animals were anesthetized with equitesin (chlornembutal, 0.3 ml/kg, intraperitoneal), and 6-OHDA was bilaterally infused (6 μg in 1 μl of cerebrospinal fluid added to 0.2% ascorbic acid) using a 27-gauge needle attached to a 10 μl syringe (Hamilton, USA). The following coordinates were used for the SNpc: anterior/posterior, –5.0 mm from bregma; medial/lateral, ± 2.1 mm from the midline; dorsal/ventral, 8 mm from the skull [24]. To prevent reflux of the neurotoxin, the needle was kept at the infusion site for 2 min after completion of the procedure. The sham groups also underwent the same procedure but received artificial cerebrospinal fluid instead of 6-OHDA.

Open field test

The apparatus consisted of a circular, 100 cm diameter, 45 cm high arena, with the floor divided into 19 units. The animals were gently placed always in the same unit of the open field and allowed to freely explore the arena for 5 min. Two motor parameters were recorded throughout this test: locomotion frequency (i.e., the number of crossings from one unit to another) and rearing frequency (i.e., the number of times the animals stood on their hind paws). The open field was washed with a 5% water-ethanol solution before the behavioral tests to eliminate possible bias caused by odors left by previous rats. This test was performed according to Experiment 1, as explained in experimental design, 24 h and 21 days after stereotaxic surgery.

Modified forced swim test

The modified forced swim test is often used to screen antidepressant drugs [25]. This test was performed 22 days after stereotaxic surgery. The animals were subjected to a 15 min training session on day 21. They were placed in a tank of 25 cm diameter and 60 cm height that contained water at a temperature of 24 ± 1 ºC and depth of 25 cm. Twenty-four hours prior to the training session, the rats were subjected to the forced swim test for 5 min. The entire experiment was filmed, and immobility, climbing and swimming parameters were analyzed and quantified. After testing each animal, the water was changed to avoid any influence. This procedure is a modification [26] of the method proposed by Porsolt *et al.* [27].

Sucrose preference test

To verify anhedonia-like behavior, the sucrose preference test was performed [28]. The animals were transferred to individual housing cages with free access to food. Each rat was provided with two bottles of water on the extreme sides of the cage during the 24 h training phase to adapt the rats to drinking from two bottles. After training, one bottle was randomly switched to contain 1% sucrose solution, the bottles were weighed before being presented to the rats and after 24 h. Fluid consumption was quantified by calculating the difference in the weights of the bottles after 24 h (% sucrose preference = sucrose intake × 100/ total intake). The experiment was conducted before surgery to obtain baseline values and subsequently performed weekly until day 21, always between 9:00 AM and 10:00 AM. Animals with sucrose preference < 75% in the baseline assessment were discarded from the study.

Determination of dopamine, norepinephrine, serotonin and metabolite concentrations

We used reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection to measure the levels of DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), NE, dihydroxyphenylglycol (DHPG), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus and striatum. On day 21, the rats were decapitated, and encephalic structures were dissected and stored at −80 ºC until processed for neurochemical quantification. The HPLC system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 mm x 4.6 mm inner diameter, 4 μm particle size) fitted with a 4 mm x 3.0 mm pre-column (Security Guard Cartridges Fusion-RP), an electrochemical detector (ESA Coulochem III Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV, a dual-electrode analytical cell (ESA 5011A), and a LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 μl loop. A 25 ºC control temperature was maintained inside the column. The cell contained two chambers in series. Each chamber

included a porous graphite coulometric electrode, a double counter electrode and a double reference electrode. Oxidizing potentials were set at 100 mV for the first electrode and 450 mV for the second electrode. The tissue samples were homogenized with an ultrasonic cell disrupter (Sonic) and 0.1 M perchloric acid that contained 0.02% sodium metabisulfite and an internal standard. After centrifugation at 10,000 x G for 30 min at 4 ºC, 20 µl of the supernatant was injected into the chromatograph. The mobile phase, with a flow rate of 1 ml/min, had the following composition: 20 g citric acid monohydrate (Merck), 200 mg octane-1-sulfonic acid sodium salt (Merck), 40 mg ethylenediaminetetraacetic acid (EDTA; Sigma), and 900 ml HPLC-grade water. The pH of the buffer running solution was adjusted to 4.0, and the solution was filtered through a 0.45 µm filter. Methanol (Merck) was added to give a final composition of 10% methanol (v/v). The neurotransmitter and metabolite concentrations were calculated using standard curves that were generated by determining the ratios between three different known amounts of the internal standard in triplicate. The units are expressed as µg/g of wet weight.

Immunohistochemistry

Midbrain dopaminergic cells were assessed using tyrosine hydroxylase (TH) immunohistochemical stain according to Reksidler *et al.* [29]. After the animals were anesthetized with thiopental, saline was intracardially infused, followed by a 4% paraformaldehyde fixative solution. Subsequently, the brains were removed and immersed in fixative solution for 3 days at 4 ºC. Paraformaldehyde was changed daily until the third day of the procedure. During 3 days, the brains were placed in a 30% sucrose solution for 48 h. Four sections of 30 µm thickness were cut on a cryostat in the coronal plane covering about 360 µm (–4.92 to –5.28 from bregma) of the midbrain [24]. These coordinates correspond to the maximal extent of the dopaminergic neurons within the SNpc. The samples were placed on a plate with wells, and each well contained four slices of the brain from each animal. The slices were washed three times with 0.1 M phosphate-buffered saline (PBS) solution for 10 min each. Endogenous peroxidase was blocked with a H_2O_2 + distilled water solution for 10 min. The sections were washed again with 0.1 M PBS for 10 min, and the reaction was stopped with blocking buffer (30 ml of 0.1 M PBS, 84 µl Triton X-100, and 450 µl normal goat serum). After blocking, the slices were incubated overnight in a solution that contained the primary antibody TH. The next day, the sections were washed five times for 5 min each with 0.1 M PBS solution and subsequently incubated for 2 h with the secondary antibody biotin. After 2 h, the slices were washed again three times for 10 min each with 0.1 M PBS and then incubated in a solution of Complex AB (five drops of Reagent A, 30 ml of PBS + Triton, and 5 drops of Reagent B) for 2 h. The slices were washed again with 0.1 M PBS for 10 min, and the samples were incubated with DAB (3,3-diaminobenzidine) for 6-8 min. Finally, the slices were washed with 0.1 M PBS (three times for 5 min each and three times for 10 min each). The sections were mounted on gelatin-coated slides and dried for 48 h. The slices were dehydrated in alcohol and cleared in xylene (5 min for each solution). The slides were then covered with entellan, coverslipped, and analyzed using an optical microscope by comparing the number of TH-positive cells in the SNpc in all of the groups.

Statistical analysis

Differences between groups in the open field test and forced swim test were analyzed using one-way analysis of variance (ANOVA) followed by the Newman-Keuls *post hoc* test. The sucrose preference test data were analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test. The neurochemical and histological data were analyzed using one-way ANOVA followed by the Newman-Keuls test. The data are expressed as mean ± standard error of the mean (SEM). The level of significance was set at $p \leq 0.05$.

RESULTS

Open field test

With regard to locomotor activity (Table 1) 24 h after stereotaxic surgery, rearing frequency ($F_{3,40} = 7.769$, $p = 0.0003$) and locomotion frequency ($F_{3,40} = 11.49$, $p < 0.0001$) were reduced in the 6-OHDA groups compared with sham rats ($p < 0.01$). In the second measure performed on day 21,

Table 1. Motor behavior alterations determined 1 and 21 days after 6-OHDA exposure.

	Group	Locomotion frequency	Rearing frequency
Day 1	Sham-vehicle	99.27 ± 7.58	21.27 ± 1.95
	Sham-curcumin	110.1 ± 11.52	18.36 ± 2.03
	6-OHDA-vehicle	49.42 ± 14.54*	9.5 ± 3.19*
	6-OHDA-curcumin	28.9 ± 9.38**	6.5 ± 2.42*
Day 21	Sham-vehicle	79.82 ± 9.02	15.45 ± 2.44
	Sham-curcumin	67.18 ± 13.76	15.22 ± 2.13
	6-OHDA-vehicle	69.20 ± 10.21	16.78 ± 3.11
	6-OHDA-curcumin	71.63 ± 12.7	16.11 ± 1.95

The data are expressed as mean ± SEM (n = 10-12/group). *$p < 0.01$, **$p < 0.001$, compared with sham groups (ANOVA followed by Newman-Keuls test).

no differences in rearing frequency ($F_{3,36} = 0.07918$, $p = 0.9709$) or locomotion frequency ($F_{3,36} = 0.2508$, $p = 0.8603$) were observed between groups.

Modified forced swim test

The 6-OHDA-vehicle group showed an increase in immobility time ($F_{3,30} = 7.237$, $p = 0.001$) compared with the sham groups (sham-vehicle, $p < 0.05$; sham-curcumin, $p < 0.01$). The 6-OHDA-curcumin group exhibited a reduction of this parameter compared with the 6-OHDA-vehicle group ($p < 0.01$; Fig. 2A). The swimming parameters ($F_{3,30} = 8.056$, $p = 0.0004$) showed that the 6-OHDA-curcumin group exhibited an increased swimming time compared with the 6-OHDA-vehicle group ($p < 0.01$). A reduction of swimming time was observed in the 6-OHDA-vehicle group compared with the sham-vehicle group ($p < 0.05$) and sham-curcumin group ($p < 0.001$). In contrast, the sham-curcumin group exhibited an increase in this parameter compared with the sham-vehicle group ($p < 0.05$; Fig. 2B). Climbing time ($F_{3,30} = 1.667$, $p = 0.1952$) did not significantly differ between groups (Fig. 2C).

Sucrose preference test

As shown in Fig. 3, sucrose consumption in the 6-OHDA-vehicle group decreased compared with the 6-OHDA-curcumin, sham-vehicle and sham-curcumin groups on day 14 ($p < 0.001$). Sucrose preference in the 6-OHDA-vehicle group remained lower than in the sham groups ($p < 0.01$) on day 21. No difference was observed between the sham groups and 6-OHDA-curcumin group at any of the time-points.

Determination of dopamine, norepinephrine, serotonin and metabolite concentrations

Striatal DA concentrations ($F_{3,37} = 9.805$, $p = 0.0002$) decreased in the 6-OHDA-vehicle group compared with both sham groups ($p < 0.001$). The 6-OHDA-curcumin group exhibited an increase in DA concentrations compared with the 6-OHDA-vehicle group ($p < 0.01$). However, DA levels in the striatum in the 6-OHDA-curcumin group decreased compared with the sham groups ($p < 0.01$). A significant difference was observed between the sham groups ($p < 0.05$), when animals treated with curcumin exhibited an increase in DA concentrations (Table 2). The concentrations of DOPAC ($F_{3,37} = 10.60$, $p < 0.0001$) and HVA ($F_{3,37} = 9.805$, $p = 0.0002$) in the striatum exhibited the same profile. In the 6-OHDA-vehicle group, the metabolite concentrations were reduced compared with the sham groups ($p < 0.001$). Interestingly, no difference was found between the 6-OHDA-vehicle and 6-OHDA-curcumin groups. Reductions of DOPAC and HVA concentrations were observed in the 6-OHDA-curcumin group compared with the sham groups ($p < 0.05$; Table 2).

The 6-OHDA-vehicle group exhibited a significant reduction of hippocampal NE levels ($F_{3,37} = 11.58$, $p < 0.0001$) compared with the sham groups ($p < 0.001$; Table 3). The 6-OHDA-curcumin group exhibited a decrease in NE concentration in the hippocampus compared with the sham-vehicle group ($p < 0.01$) and sham-curcumin group ($p < 0.001$).

The concentrations of 5-HT ($F_{3,37} = 0.6210$, $p = 0.6059$) and 5-HIAA ($F_{3,37} = 0.3746$, $p = 0.7718$)

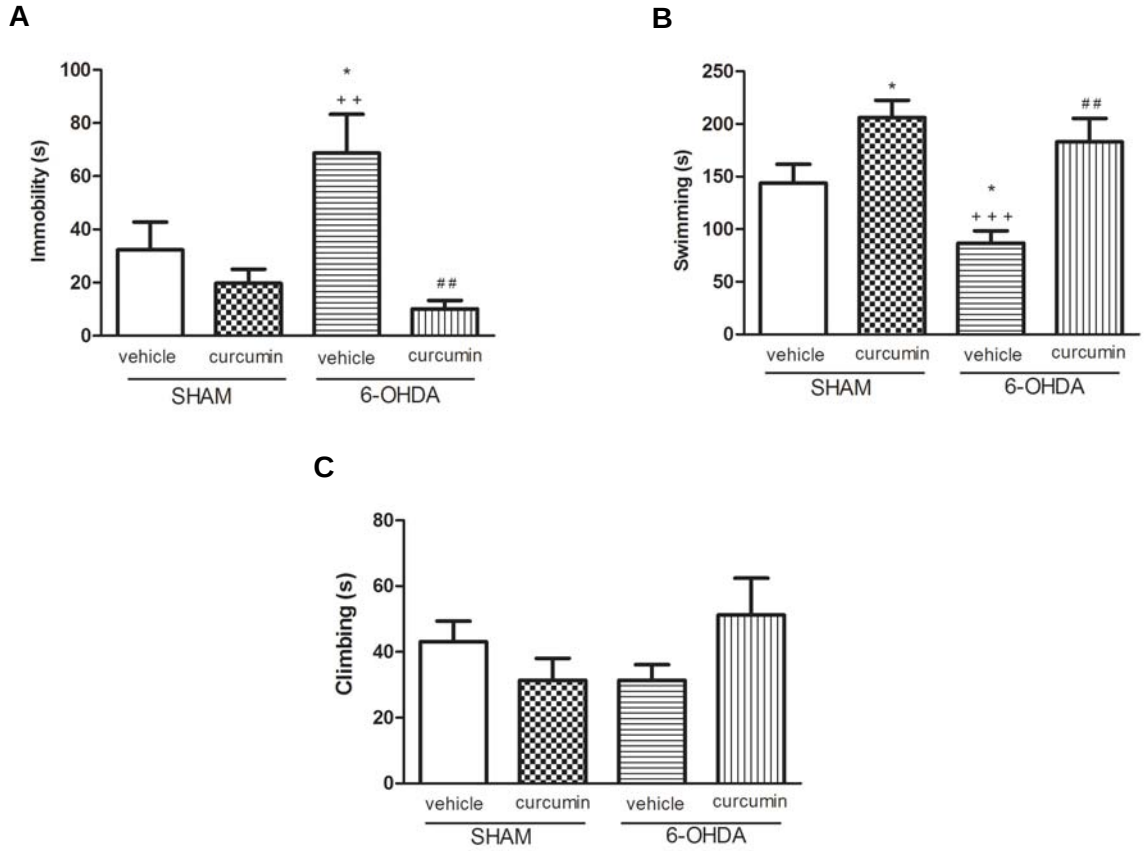

Fig. 2. Antidepressant-like effect of curcumin (30 mg/kg, p.o.) and depressive-like behavior caused by 6-OHDA injection in the forced swim test. (A) Immobility. (B) Swimming. (C) Climbing. The data were obtained 22 days after neurotoxin exposure. The data are expressed as mean ± SEM (n = 10-12/group). *$p < 0.05$, compared with sham-vehicle group; $^{\#\#}p < 0.01$, compared with 6-OHDA-vehicle group; $^{++}p < 0.01$, $^{+++}p < 0.001$, compared with sham-curcumin group (ANOVA followed by Newman-Keuls test).

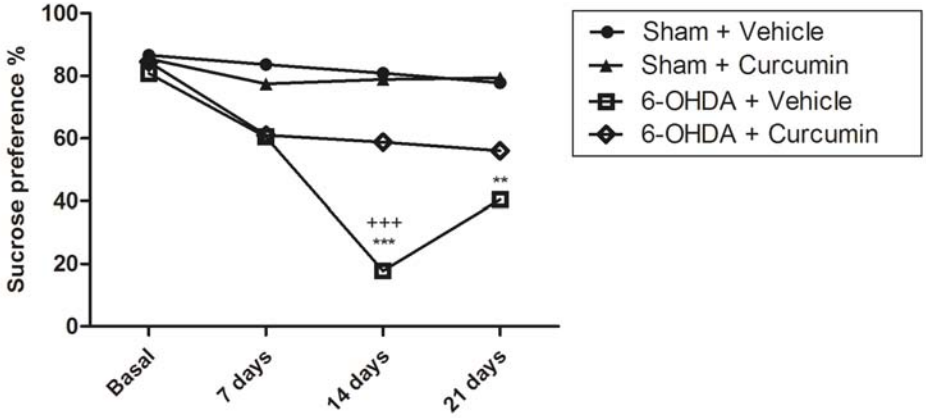

Fig. 3. Antidepressant-like effect of curcumin (30 mg/kg, p.o.) and anhedonic-like behavior caused by 6-OHDA injection, reflected by the percentage of sucrose preference in each group at different time-points. The data are expressed as the mean (n = 12-14/group). **$p < 0.01$, ***$p < 0.001$, compared with sham groups; $^{+++}p < 0.001$, compared with 6-OHDA-curcumin group (two-way ANOVA followed by Bonferroni test).

Table 2. Effect of curcumin on dopamine, DOPAC, and HVA levels (µg/g) in the striatum 22 days after 6-OHDA exposure.

Group	Dopamine	DOPAC	HVA
Sham-vehicle	7.18 ± 0.23	7.079 ± 0.6	0.44 ± 0.02
Sham-curcumin	$8.68 \pm 0.58^{+}$	7.085 ± 0.54	0.44 ± 0.02
6-OHDA-vehicle	$2.75 \pm 0.51^{+++}$	$3.05 \pm 0.67^{+++}$	$0.26 \pm 0.02^{+++}$
6-OHDA-curcumin	4.90 ± 0.68 **$^{,++}$	$4.67 \pm 0.49^{+}$	$0.33 \pm 0.03^{+}$

The data are expressed as mean ± SEM (n = 10-12/group). **$p < 0.01$, compared with 6-OHDA-vehicle group; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, compared with sham-vehicle group (ANOVA followed by Newman-Keuls test).

Table 3. Effect of curcumin on 5-HT, 5-HIAA, NE, and DHPG levels (µg/g) in the hippocampus 22 days after 6-OHDA exposure.

Group	Serotonin	5-HIAA	Norepinephrine	DHPG
Sham-vehicle	0.39 ± 0.04	0.89 ± 0.03	0.54 ± 0.04	0.88 ± 0.06
Sham-curcumin	0.32 ± 0.03	0.81 ± 0.05	0.58 ± 0.04	0.85 ± 0.06
6-OHDA-vehicle	0.34 ± 0.04	0.83 ± 0.04	$0.25 \pm 0.05^{+++}$	$0.51 \pm 0.09^{++}$
6-OHDA-curcumin	0.33 ± 0.05	0.85 ± 0.10	$0.31 \pm 0.05^{++}$	0.80 ± 0.08*

The data are expressed as mean ± SEM (n = 10-12/group). *$p < 0.05$, compared with 6-OHDA-vehicle group; $^{++}p < 0.01$, $^{+++}p < 0.001$, compared with sham groups (ANOVA followed by Newman-Keuls test).

in the hippocampus were not significantly different between groups (Table 3).

Immunohistochemistry

As shown in Fig. 4, the sham groups exhibited strong expression of TH-immunoreactive neurons in the SNpc. The 6-OHDA-vehicle group exhibited a reduction in the expression of these neurons. The number of TH-immunoreactive neurons in the 6-OHDA-vehicle group, calculated by comparing the optical density of each image, significantly differed from the sham-vehicle group ($p < 0.01$) and sham-curcumin group ($p < 0.001$; $F_{3,83} = 8.903$, $p < 0.0001$). Fig. 4 also shows that the 6-OHDA-curcumin group exhibited a slight reduction of TH-positive neurons. Moreover, the 6-OHDA-vehicle group exhibited a significant decrease in TH-immunoreactive neurons in the SNpc compared with the 6-OHDA-curcumin group ($p < 0.05$).

DISCUSSION

The death of dopaminergic neurons in the SNpc in animals injured with 6-OHDA resulted in a depression-like behavior and a state of anhedonia. However, treatment with curcumin (30 mg/kg, p.o.) for 21 days had a neuroprotective effect, reducing the depression-like behavior and the state of anhedonic, and increasing monoamines levels.

To discard the possible influence of baseline locomotor activity before testing the animals in behavioral models of depression, we performed the open field test. Hypolocomotion observed 24 h after surgery in rats that received 6-OHDA was not reversed by a single dose of curcumin. The decrease in motor behavior is likely attributable to neuronal loss observed 24 h after neurotoxin exposure. This reduction of locomotor activity was reversed in both lesioned groups on day 21, ensuring that motor influences did not affect the results obtained in the behavior models of depression.

To evaluate the antidepressant-like effect of curcumin, the forced swim test and sucrose preference test were performed to determine whether curcumin can reverse the depression-like behavior induced by the neurotoxin. Sucrose preference in the

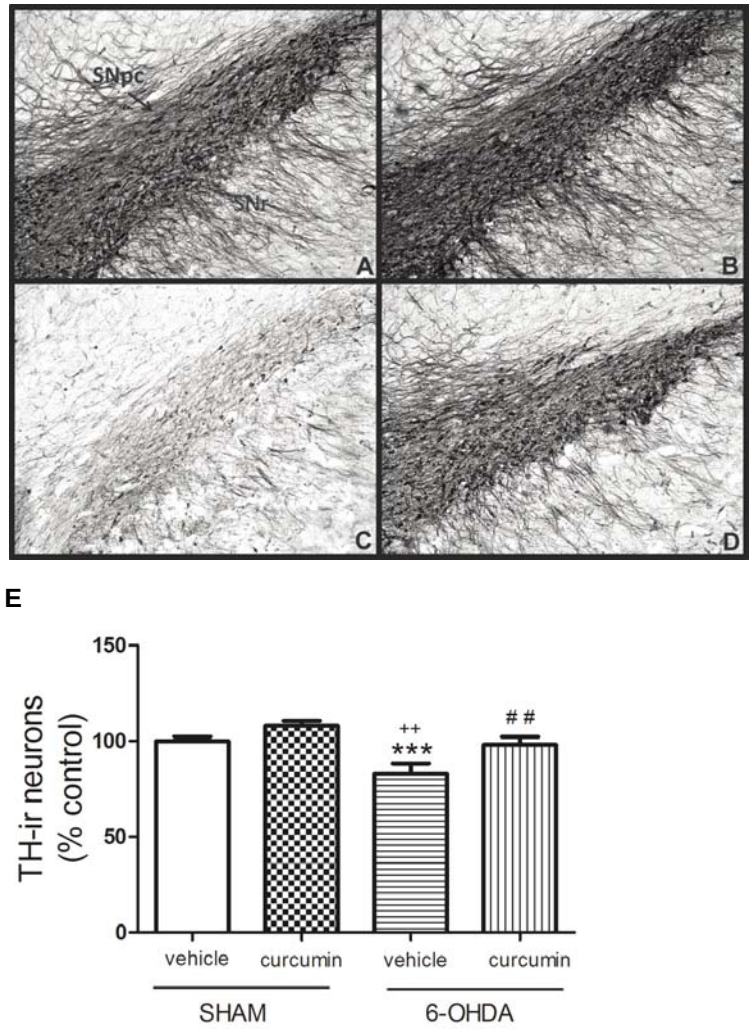

Fig. 4. Effects of curcumin on TH-positive neurons in the SNpc. Immunohistochemistry figures show the quantity of TH-positive neurons in the SNpc in all of the groups 22 days after 6-OHDA infusion. (A) Sham-vehicle. (B) Sham-curcumin. (C) 6-OHDA-vehicle. (D) 6-OHDA-curcumin. (E) Percentage of TH-positive neurons, determined by calculating the optical density. The data are expressed as mean ± SEM (n = 3-4/group). $^{\#\#}p < 0.01$, compared with 6-OHDA-vehicle group; $^{++}p < 0.01$, compared with sham-vehicle group; $^{***}p < 0.001$, compared with sham-curcumin group (ANOVA followed by Newman-Keuls test). Substantia nigra *pars reticulata* (SNr).

6-OHDA-vehicle group was lower than in the control groups, indicating an anhedonic-like state caused by the neurotoxin in these rats. These results are consistent with previous studies, in which the neurotoxin provoked dopaminergic neuron damage and led to an anhedonic-like state [5, 30]. Rats that received 6-OHDA and treated with curcumin did not exhibit a reduction of sucrose preference compared with control rats. Moreover, we observed an increase in sucrose preference in these animals on day 14, compared with the 6-OHDA-vehicle group. These data indicate that curcumin can reverse the anhedonic-like state induced by 6-OHDA. Previous studies that used other models of depression-like behavior demonstrated curcumin's ability to reverse anhedonic-like states induced by chronic unpredictable mild stress and chronic corticosterone administration [14, 31, 32]. The present study further shows a similar effect of curcumin in the 6-OHDA-induced model of anhedonia.

In the forced swim test, an increase in immobility time is related to "behavioral despair" that can be extrapolated to a condition similar to human

depression [33]. Bilateral infusion of 6-OHDA in the SNpc, similar to a previous study [5], increased the immobility time and reduced the swimming time. In contrast, the 6-OHDA-curcumin group exhibited a reduction in immobility time and increase in swimming time compared with the 6-OHDA-vehicle group. Interestingly, in sham animals, the dose of curcumin used did not exert antidepressant-like activity in the forced swim test, in which no significant difference was observed in immobility time between the sham-vehicle and sham-curcumin groups. This result is consistent with the lack of changes in monoamine content observed in the hippocampus. These results indicate that the effect of curcumin in 6-OHDA-lesioned rats was not attributable to specific effects. However, a significant effect on swimming time was found. This may have been caused by a threshold effect of the curcumin dose used (i.e., although it increased swimming behavior, this was not sufficient to decrease immobility).

To corroborate the behavioral test results, neurochemical and immunohistochemical analyses were performed. In the analysis of the hippocampus, NE concentrations were more affected by the neurotoxin than 5-HT concentrations. The 6-OHDA-vehicle group exhibited a decrease in NE and its metabolite DHPG compared with controls. The neurotoxin group treated with curcumin exhibited a decrease in NE levels compared with controls. However, DHPG levels were significantly higher in the 6-OHDA-curcumin group than in the 6-OHDA-vehicle group and did not significantly differ from that in the sham groups. The concentrations of 5-HT and its metabolite in the hippocampus were statistically equal among groups. In other animal models of depression, such as chronic unpredictable mild stress and olfactory bulbectomy [31, 34], curcumin altered serotoninergic function and concentration. In the present 6-OHDA model, curcumin did not alter 5-HT levels in the hippocampus. The curcumin groups did not exhibit an increase in 5-HT concentrations, but the 6-OHDA-vehicle group also did not exhibit a decrease in 5-HT concentrations. With regard to striatal DA, the 6-OHDA-vehicle group exhibited a large decrease in the levels of this neurotransmitter compared with controls. This decrease was also apparent when we measured the DA metabolites DOPAC and HVA, indicating the death of dopaminergic neurons. The same conclusion may be drawn from the immunohistochemical analyses, in which the 6-OHDA-vehicle group exhibited a significant reduction in the expression of TH-immunoreactive neurons. The 6-OHDA-curcumin group exhibited an increase in DA concentrations compared with the 6-OHDA-vehicle group, also supporting the histological analyses and suggesting a neuroprotective effect of curcumin. DOPAC and HVA levels in the 6-OHDA-curcumin group were also higher, coinciding with the DA concentrations, but this increase did not significantly differ from the 6-OHDA-vehicle group.

Curcumin may be considered a multi-target drug that exerts its neuroprotective and antidepressant-like effects in the 6-OHDA model through numerous mechanisms [8]. Neuronal death induced by 6-OHDA might be prevented through anti-inflammatory and antioxidant mechanisms [35-37]. Curcumin can exert protective effects by inhibiting glial activation and reducing the expression of pro-apoptotic proteins, such as nuclear factor-κB and caspase-3 [23, 37, 38]. Although the antidepressant-like effect of curcumin appears to be mainly related to alterations in serotoninergic function in animal models of depression [39], its antidepressant-like effect in the 6-OHDA-induced model of PD may be modulated by a dopaminergic mechanism. Previous studies have correlated curcumin treatment with an increase in the levels of DA and its metabolites in both PD and depression models [8, 15, 22, 23, 34, 40-42]. Imaging studies found a strong correlation between impairment of the DA system and depression symptoms in PD patients [43, 44]. Moreover, PD-related depression appears to be correlated with dopaminergic pathways. Studies of 5-HT in PD have provided inconclusive results. Patients with PD present with a reduction of DA pathway activity associated with reward and mood systems. These data support the hypothesis that the dopaminergic system is mainly involved in the etiology of PD-related depression [6, 45].

CONCLUSION

Altogether, the present results confirmed our hypothesis that curcumin exerts antidepressant-like effects in PD-related depression. Using two behavior models of depression, curcumin treatment reversed the depression-like behavior observed in

animals injured with 6-OHDA, confirming the antidepressant-like effect of curcumin in this model. The behavioral results were corroborated by the neurochemical and immunohistological analyses. The present study indicates that the antidepressant-like effect of curcumin in this model is more related to the preservation of dopaminergic function compared with other monoamines.

ACKNOWLEDGEMENTS

This work was supported by grants from CNPq, CAPES and REUNI that had no further role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication. MABFV and RA are recipients of CNPq fellowship.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

REFERENCES

1. Gerlach, M. and Riederer, P. 1996, Journal of Neural Transmission, 103, 987.
2. Dauer, W. and Przedborski, S. 2003, Neuron, 39, 889.
3. Hornykiewicz, O. 2006, Journal of Neural Transmission Supplement, 70, 9.
4. Chaudhuri, K. R., Healy, D. G. and Schapira, A. H. V. 2006, Lancet Neurology, 5, 235.
5. Santiago, R. M., Barbiero, J. K., Lima, M. M. S., Dombrowski, P. A., Andreatini, R. and Vital, M. A. B. F. 2010, Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34, 1104.
6. Aarsland, D., Pahlhagen, S., Ballard, C. G., Ehrt, U. and Svenningsson, P. 2012, Nature Reviews Neurology, 8, 35.
7. Wolters, E. C. 2008, Journal of the Neurological Sciences, 266, 197.
8. Hurley, L. L. and Tizabi, Y. 2013, Neurotoxicity Research, 23, 131.
9. Zunszain, P. A., Hepgul, N. and Pariante, C. M. 2013, Current Topics in Behavioural Neurosciences, 14, 135.
10. Gao, H. M., Jiang, J., Wilson, B., Zhang, W., Hong, J. S. and Liu, B. 2002, Journal of Neurochemistry, 81, 1285.
11. Leentjens, A. F., Scholtissen, B., Vreeling, F. W. and Verhey, F. R. 2006, Neuropsychopharmacology, 31, 1009.
12. Skapinakis, P., Bakola, E., Salanti, G., Lewis, G., Kyritsis, A. P. and Mavreas, V. 2010, BMC Neurology, 10, 49.
13. Jaques, J. A. S., Rezer, J. F. P., Gonçalves, J. F., Spanevello, R. M., Gutierres, J. M., Pimentel, V. C., Thomé, G. R., Morsch, V. M., Schetinger, M. R. and Leal, D. B. 2011, Cell Biochemestry and Function, 29, 703.
14. Lopresti, A. L., Hood, S. D. and Drummond, P. D. 2012, Journal of Psychopharmacology, 26, 1512.
15. Xu, Y., Ku, B. S., Yao, H. Y., Lin, Y. H., Ma, X., Zhan, Y. H. and Li, X. J. 2005, European Journal of Pharmacology, 518, 40.
16. Kulkarni, S. K., Dhir, A. and Akula, K. K. 2009, Scientific World Journal, 9, 1233.
17. Hurley, L., Akinfiresoye, L., Nwuliab, E., Kamiyac, A., Kulkarni, A. A. and Tizabi, Y. 2013, Behavioural Brain Research, 239, 27.
18. Yu, S., Zheng, W., Xin, N., Chi, Z. H., Wang, N. Q., Nie, Y. X., Feng, W. Y. and Wang, Z. Y. 2010, Rejuvenation Research, 13, 55.
19. Rajeswari, A. and Sabesan, M. 2008, Inflammopharmacology, 16, 96.
20. Pandey, N., Strider, J., Nolan, W. C., Yan, S. X. and Galvin, J. E. 2008, Acta Neuropathologica, 115, 479.
21. Ahmad, B. and Lapidus, L. J. 2012, Journal of Biological Chemistry, 287, 9193.
22. Agrawal, S. S., Gullaiya, S., Dubey, V., Singh, V., Kumar, A., Nagar, A. and Tiwari, P. 2012, Cardiovascular Psychiatry and Neurology, 2012, 942.
23. Tripanichkul, W. and Jaroensuppaperch, E. O. 2012, International Journal of Neuroscience, 122, 263.
24. Paxinos, G. and Watson, C. 2005, The rat brain in stereotaxic coordinates: the new coronal set, (5th ed.), New York, Academic Press.
25. Cryan, J. F., Markou, A. and Lucki, I. 2002, Trends in Pharmacological Sciences, 23, 238.
26. Reneric, J. P., Bouvard, M. and Stinus, L. 2002, Behavioural Brain Research, 136, 521.
27. Porsolt, R. D., Anton, G., Blavet, N. and Jalfre, M. 1978, European Journal of Pharmacology, 47, 379.

28. Papp, M., Willner, P. and Muscat, R. 1991, Psychopharmacology (Berl.), 104, 255.
29. Reksidler, A. B., Lima, M. M., Zanata, S. M., Machado, H. B., Da Cunha, C., Andreatini, R., Tufik, S. and Vital, M. A. B. F. 2007, European Journal of Pharmacology, 560, 163.
30. Tadaiesky, M. T., Dombrowski, P. A., Figueiredo, C. P., Cargnin-Ferreira, E., Da Cunha, C. and Takahashi, R. N. 2008, Neuroscience, 156, 830.
31. Li, Y. C., Wang, F. M., Pan, Y., Qiang, L. Q., Cheng, G., Zhang, W. Y. and Kong, L. D. 2009, Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33, 435.
32. Huang, Z., Zhong, X. M., Li, Z. Y., Feng, C. R., Pan, A. J. and Mao, Q. Q. 2011, Neuroscience Letters, 493, 145.
33. Malkesman, O. and Weller, A. 2009, Prog. Neurobiol., 88, 153.
34. Xu, Y., Ku, B. S., Yao, H. Y., Lin, Y. H., Ma, X., Zhang, Y. H. and Li, X. J. 2005, Pharmacology Biochemistry and Behavior, 82, 200.
35. Sandur, S. K., Ichikawa, H., Pandey, M. K., Kunnumakkara, A. B., Sung, B., Sethi, G. and Aggarwal, B. B. 2007, Free Radical Biology and Medicine, 43, 568.
36. Jurenka, J. S. 2009, Alternative Medicine Review, 14, 141.
37. Wang, J., Du, X. X., Jiang, H. and Xie, J. X. 2009, Biochemical Pharmacology, 78, 178.
38. Arora, V., Kuhad, A., Tiwari, V. and Chopra, K. 2011, Psychoneuroendocrinology, 36, 1570.
39. Wang, R., Xu, Y., Wu, H. L., Li, Y. B., Li, Y. H., Guo, J. B. and Li, X. J. 2008, European Journal of Pharmacology, 578, 43.
40. Pan, J., Li, H., Ma, J. F., Tan, Y. Y., Xiao, Q., Ding, J. Q. and Chen, S. D. 2012, Translational Neurodegeneration, 1, 16.
41. Du, X. X., Xu, H. M., Jiang, H., Song, N., Wang, J. and Xie, J. X. 2012, Neuroscience Bulletin, 28, 253.
42. Kulkarni, S. K., Bhutani, M. K. and Bishnoi, M. 2008, Psychopharmacology, 201, 435.
43. Brooks, D. J. and Piccini, P. 2006, Biological Psychiatry, 59, 908.
44. Rektorova, I., Srovnalova, H., Kubikova, R. and Prasek, J. 2008, Movement Disorders, 23, 1580.
45. Remy, P., Doder, M., Lees, A., Turjanski, N. and Brooks, D. 2005, Brain, 128, 1314.