

# Neuroprotective effect of the systemic delivery of (-) nicotine in hemiparkinsonian rats

✉ Lisette Blanco<sup>1</sup>, Lourdes Lorigados<sup>1</sup>, Rocío García<sup>1</sup>, Lisis Martínez<sup>1</sup>, Nancy Pavón<sup>1</sup>, María E González<sup>1</sup>, Teresa Serrano<sup>1</sup>, Vivian Blanco<sup>2</sup>

<sup>1</sup>Centro Internacional de Restauración Neurológica, CIREN  
Ave. 25 No. 15805 e/ 158 y 160, Playa, Ciudad de La Habana, Cuba  
E-mail: lisette.blanco@infomed.sld.cu

<sup>2</sup>Centro Comunitario de Salud Mental  
Calle 1<sup>ra</sup>. No. 19606 e/ 10 y 12, Boyeros, Ciudad de La Habana, Cuba

RESEARCH

## ABSTRACT

The detection of lower incidences of Parkinson's disease (PD) among smokers has sparked a renewed interest in the administration of (-) nicotine as a tool for the manipulation of the cholinergic system in experimental models of parkinsonism. This work evaluated five groups of Wistar rats (1: Healthy rats (n = 11), 2: Rats with lesions in the *substantia nigra pars compacta* (SNc) (n = 10), 3: Rats with SNc lesions, treated with (-) nicotine (n = 18), 4: Rats with false SNc lesions, obtained by treatment with physiological saline solution (PSS) (n = 10), 5: Rats with SNc lesions, treated with PSS (n = 10); measuring motor coordination and asymmetry, the extracellular concentrations of glutamate (Glu) and  $\gamma$ -aminobutyric acid (GABA) in the pedunculopontine nucleus (PPN), cell survival at the SNc, and the striatal expression of several neurotrophic factors. SNc-injured rats treated with (-) nicotine performed better and had smaller average numbers of errors at the largest diameter bridges when compared to untreated hemiparkinsonian rats. The treatment with (-) nicotine decreased the extracellular concentration of Glu and GABA at the PPN, attenuated motor asymmetry, promoted a higher striatal expression of brain-derived neurotrophic factor and diminished the loss of dopaminergic cells from the SNc. The results suggest a neuroprotective effect for (-) nicotine in the administration scheme employed in this study.

Keywords: (-) nicotine, basal ganglia, *substantia nigra compacta*, pedunculopontine nucleus

*Biotecnología Aplicada* 2008;25:126-134

## RESUMEN

**Efecto neuroprotector de la administración sistémica de (-) nicotina en ratas hemiparkinsonizadas.** La manipulación del sistema colinérgico en el parkinsonismo experimental, a través de la administración de (-) nicotina, adquiere gran importancia a partir del hallazgo de una menor incidencia de la enfermedad de Parkinson (EP) en sujetos fumadores. Se evaluaron cinco grupos de ratas Wistar: 1. Ratas sanas (n = 11), 2. Ratas lesionadas en la *substantia nigra compacta* (SNc) (n = 10), 3. Ratas tratadas con (-) nicotina y lesionadas en la SNc (n = 18), 4. Ratas con falsa lesión de la SNc por inyección de solución salina fisiológica (SSF) (n = 10), 5. Ratas tratadas con SSF y lesionadas en la SNc (n = 10). Se evaluaron la coordinación y la asimetría motora, las concentraciones extracelulares de glutamato (Glu) y ácido  $\gamma$ -aminobutírico (GABA) en el núcleo pedunculopontino (NPP), la supervivencia de células en la SNc y la expresión estriatal de distintos factores neurotróficos. Las ratas tratadas con (-) nicotina y lesionadas en la SNc mostraron una mejor ejecución y un menor número promedio de errores en los puentes de mayores diámetros en comparación con las ratas hemiparkinsonizadas no tratadas con la droga. El tratamiento con (-) nicotina disminuyó las concentraciones extracelulares de Glu y GABA en el NPP, atenuó la asimetría motora de las ratas, promovió más expresión estriatal del factor neurotrófico derivado del cerebro y hubo menos pérdida de células dopaminérgicas en la SNc. El esquema de administración de (-) nicotina en este estudio sugiere un efecto neuroprotector.

Palabras clave: (-) nicotina, ganglios basales, *substantia nigra compacta*, núcleo pedunculopontino

## Introduction

The discovery of dopaminergic deficiencies as the distinctive feature of Parkinson's disease (PD) paved the way for the successful pharmacological treatment of this disorder through the administration of a dopamine precursor, L-dihydroxyphenylalanine (L-DOPA) [1, 2]. However, it is now known that long-term levodopa therapy results in a number of side effects, such as involuntary movements and fluctuations in the motor response [3]. Different pharmacological alternatives have been tested with the aim of counterbalancing the neurochemical disequilibrium of the basal ganglia (BG) induced by the degeneration of the nigrostriatal pathway [4], and a significant emphasis has recently been placed on the study of neuropro-

TECTIVE drugs that prevent the death of nigral cells or decelerate the degenerative course of this disease [5-7]. In this context, the discovery of lower incidences of PD among smokers [8] led to the investigation of (-) nicotine as a potential neuroprotective drug that might prevent the degeneration of the dopaminergic cells of the *substantia nigra compacta* (SNc). Nicotine is a natural alkaloid from the plant *Nicotiana tabacum*, with well-known effects on the central and peripheral nervous system [9]. Although the mechanism behind the protective effect of nicotine on dopaminergic cells has not been elucidated, it is hypothesized to be mediated, among others, by the synthesis and release of neurotrophic factors, the release of striatal dopamine

1. Hornykiewicz O. Brain neurotransmitter changes in Parkinson's disease. In: Marsden CD, Fahn S, eds. *Movement disorders*. London: Butterworth Scientific, Movement Disorders 1982;3:41-58.

2. Jankovic J. Complications and limitations of drug therapy for Parkinson's disease. *Neurology* 2000;55 (12 Suppl 6): S2-6.

3. Obeso JA, Grandas F, Vaamonde J, Luquin MR, Artieda J, Lera G, et al. Motor complications associated with chronic levodopa therapy in Parkinson's disease. *Neurology* 1989;39 (11 Suppl 2):11-9.

from cells surviving neurotoxic injury, the promotion of intracellular signaling events, and the activation of nicotinic receptors [10-14].

Although the neurochemistry of BG has been intensely studied during the last 20 years, the changes that take place in the pedunculopontine nucleus (PPN) -closely related both anatomically and functionally to the nuclei of the BG- have not received the same attention within the context of experimental models of PD [15]. It is known that the PPN is connected reciprocally to the BG, sending cholinergic and glutamatergic projections to the SNc that modulate the dopaminergic activity of nigral cells [16]. Also, the connections of the PPN to the interneuronal network of the spinal chord through the medial reticulospinal tract highlight the role of this structure on the processing of motor information [17].

The goal of this study is the evaluation of the effect of the systemic administration of (-) nicotine on the motor disorders, the survival of dopaminergic cells in the SNc, the striatal expression of neurotrophic factors (NTF) and the extracellular concentration of Glu and GABA in the PPN of hemiparkinsonian rats.

## Experimental subjects

The study used male, adult Wistar rats weighing from 200 to 250 g, provided by the Center for the Production of Laboratory Animals (CENPALAB, La Havana, Cuba). Three individuals were housed per cage throughout the experiment, with a 12 h/12 h cycle of light and darkness and water and food *ad libitum*. The experimental work followed the Guidelines for the Care, Use and Reproduction of Laboratory Animals.

## Materials and methods

### SNc lesion

The animals were anesthetized by the intraperitoneal (i.p.) administration of a chloral hydrate solution (420 mg/kg of body weight) and placed on a stereotactic surgery device for rodents (Stoelting, U.S.A). Three microliters of a neurotoxic solution containing 8 µg of 6-OHDA in 3 µL of 0.9% physiological saline solution (PSS) plus 0.5 mg/mL of ascorbic acid were injected using a flow of 1 µL/min into the right SNc, at the following stereotactic coordinates (mm) described in the Paxinos and Watson atlas [18]: AP = -0.49, L = 0.17, DV = 0.81 (according to Bregma). A control group with false SNc lesions was obtained by the administration of an identical volume of PSS at the same coordinates.

### Rotational activity

The rotational activity induced by D-amphetamine (5 mg/kg, i.p.) was studied one month after the administration of 6-OHDA, using an LE 3806 Electronic Multicounter coupled to LE 902 sensors (PanLAB, Barcelona, Spain) that measured the sense of rotation. The measurements were performed for a period of 90 minutes.

### Systemic administration of (-) nicotine

The rats received 3 subcutaneous (s.c.) injections of (-) nicotine (1 mg/kg body weight) every 30 min., after which the solution of 6-OHDA was administered.

This administration schedule was repeated at days 7, 14 and 21 after the intracerebral injection of 6-OHDA.

The false SNc lesion group was administered (-) nicotine by the same schedule, but PSS was used instead of 6-OHDA.

### Measurement of motor coordination by the transversal bridge test

The transversal bridge tests were carried out under proper conditions of silence and illumination, evaluating their results by direct observation. The rats were placed at the middle point of a bridge at a height of 60 cm from a supporting surface, equipped with escape platforms at each end. Sixty seconds were allotted for each assay, which quantified the time it took for each animal to reach any of the escape platforms (escape latency). Failure to reach either platform or falling from the bridge was scored as a latency of 60 s (fall latency). The number of errors was also simultaneously counted in each assay. An error was defined as any failed attempt to hold on to the bridge with either the extremities or the tail, any loss of balance, or any fall from the bridge before the 60 s assigned to each assay.

The bridges were 60 cm long with rectangular or circular cross-sections, used in order of increasing test complexity (rectangular large (2.5 cm diameter)-circular large (2.5 cm diameter)-rectangular small (1 cm diameter)-circular small (1 cm diameter)). The complete experiment was performed over 2 consecutive days, carrying out 3 assays per day. The final escape and fall latency figures were computed as the mean of the 6 values obtained from all assays.

### In vivo microdialysis

Two weeks after the behavioral studies, a guide cannula was surgically implanted at the coordinates (mm) corresponding to the right PPN (AP = -8.00, L = 2.00, DV = 5.40 (according to Bregma)).

The cerebral microdialysis experiments were performed 24 h after the implantation of this guide. Each rat was connected to a cerebral microdialysis infusion pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and the cannulae were then continuously perfused, at a flow of 2 µL/min, with a solution of artificial cerebrospinal fluid (aCSF) containing 125 mmol/L NaCl, 2.5 mmol/L KCl, 0.5 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, 5 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 1 mmol/L MgCl<sub>2</sub> x 6H<sub>2</sub>O, 1.2 mmol/L CaCl<sub>2</sub> and 1.2 mmol/L ascorbic acid, at a pH of 7.4 to 7.6.

The dialysates were manually collected 1 hour after beginning the process. A total of 6 samples were taken and immediately stored at -80 °C until further analysis. All the experiments were performed while the animals remained awoken.

### Biochemical evaluation. Neurotransmitter analysis. Aminoacid quantification

Aminoacid concentrations in the dialysates were measured by High Performance Liquid Chromatography (HPLC) coupled to a fluorescence detector, via derivatization with o-phthalaldehyde (OPA). Briefly, 10 µL of each sample were mixed with 10 µL of the derivatizing agent (10 mmol/L OPA dissolved into 0.1 mol/L

4. Levi MS, Brimble MA. A review of neuroprotection agents. *Curr Med Chem* 2004;11(18):2383-97.

5. Fernández-Espejo E. Pathogenesis of Parkinson's disease: prospects of neuroprotective and restorative therapies. *Mol Neurobiol* 2004;29(1):15-30.

6. Hirsch EC. How to judge animal models of Parkinson's disease in terms of neuroprotection. *J Neural Transm Suppl* 2006;70:255-60.

7. Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson's Disease: molecules to medicine. *J Clin Invest* 2006;116(7):1744-53.

8. Quik M, Kulak JM. Nicotine and nicotinic receptors: relevance to Parkinson's disease. *Neurotoxicology* 2002;23:581-94.

9. Martyn Ch, Gale Chh. Tobacco, coffee and Parkinson's disease. *BMJ* 2003;326:561-3.

10. Roceri R, Molteni R, Fumagalli F, Racagni G, Gennarelli M, Corsini G, et al. Stimulatory role of dopamine on fibroblast growth factor-2 expression in rat striatum. *J Neurochem* 2001;76:990-7.

11. Newman MB, Arendash GW, Shytle RD, Bickford PC, Tighe T, Sanberg PR. Nicotine's oxidative and antioxidant properties in CNS. *Life Sci* 2002;71:2807-20.

12. Pimlott SL, Piggot M, Owens J, Greally E, Court JA, Jaros E, et al. Nicotine acetylcholine receptor distribution in Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease and vascular dementia: *in vitro* binding study using 5-[125I]-α-85380. *Neuropsychopharmacology* 2004;29:108-16.

13. Quik M. Smoking, nicotine and Parkinson's disease. *Trends Neurosci* 2004;27:561-8.

14. Miks S, Tyndale RF. Nicotine induces brain CPY enzymes: relevance to Parkinson's disease. *J Neural Transm Suppl* 2006;70:177-80.

15. Mena-Segovia J, Bolam JP, Magill PJ. Pedunculopontine nucleus and basal ganglia: distant relatives or part of the same family?. *Trends Neurosci* 2004;27(10):585-8.

16. Rye D, Lee H, Saper C, Wainer B. Medullary and spinal efferents of the PPN tegmental nucleus and adjacent mesencephalic tegmentum in the rat. *J Comp Neurol* 1988;269:315-41.

17. Lee MS, Rinne JO, Marsden D. The pedunculopontine nucleus: its role in the genesis of movement disorders. *Yonsei Med J* 2000;41(2):167-84.

18. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press, NY. 1998

sodium tetraborate buffer containing 77 mmol/L 3-mercaptopropionic acid and 10% methanol, pH 9.3). The mixture was homogenized in a top mixer for 15 s, and the reaction was stopped after 45 s by the addition of 5% acetic acid. Twenty microliters from the resulting solution were loaded with a Hamilton syringe into a reversed-phase 80 x 4.6 mm ESA HPLC column fitted with a similar stationary phase precolumn and connected to an isocratic chromatography pump, detecting the derivatized aminoacids with a fluorescence detector (Philips PU 4027) at excitation and detection wavelengths of 340 nm and 460 nm, respectively. The chromatograms were recorded with the CHROMATEPC software application, version 4.24 (Philips). The mobile phase consisted of 0.1 mol/L NaH<sub>2</sub>PO<sub>4</sub> and 20% methanol. Each sample was analyzed in duplicate runs.

Measurement of the expression of Brain-derived neurotrophic factor (BDNF) and Glial-derived neurotrophic factor (GDNF)

#### A. Obtention of total protein preparations

After concluding all the assays and evaluations, the rats were anesthetized with chloral hydrate (480 mg/kg body weight, i.p.) and decapitated. Their brains were extracted and washed with cold 0.9% NaCl, after which the striatum (St) was dissected. The obtained tissue was frozen in liquid nitrogen, weighed and stored at -80 °C for further analysis.

Protein extraction from striatal tissue was accomplished using the TriPure technique. After homogenization in 1 mL of TriPure solution and addition of 0.2 mL of chloroform, the lower phase was isolated and treated with 0.3 mL of absolute ethanol to precipitate nucleic acids. The proteins in the resulting solution were then precipitated by the addition of 1.5 mL of isopropanol, followed by centrifugation at 4 °C, 12 000 rpm for 10 min. The pellet was washed 3 times with 0.3 mol/L guanidine thiocyanate in 95% ethanol, washed then with absolute ethanol, centrifuged under the conditions described above, and finally resuspended into 0.2 mL of 1% sodium dodecyl sulfate (SDS).

#### B. Protein quantification

Duplicate 1:100 dilutions from each protein sample were prepared in a final volume of 0.1 mL. Each dilution was then mixed with 0.1 mL of a solution prepared by mixing 5 mL of 4% bichinchoninic acid, 0.2 mL of copper sulfate and 5.2 mL of microreagent A (7 g anhydrous sodium carbonate, 1.6 g sodium hydroxide and 1.6 g sodium tartrate dissolved in 100 mL of distilled water, pH 11.25). After incubation overnight at room temperature, the absorbance of the samples at 280 nm was measured with a spectrophotometer (Shimadzu, Kyoto, Japan). The total protein concentration in the samples was determined by interpolation from a standard curve prepared with known concentrations of bovine serum albumin.

#### C. Western Blot

The immunodetection of BDNF, GDNF and  $\beta$ -actin was performed by Western blotting, using the latter molecule as a control for protein integrity. Aliquots containing 50 mg of protein were taken from each sample and electrophoresed at 80 V in a 12% poly-

acrylamide gel, together with a dual color molecular weight ladder covering the range from 10 to 250 kDa (Bio-Rad, Richmond, VA, U.S.A.). Once the protein species were resolved, they were transferred to a polyvinylidene fluoride (PVDF) membrane

(Bio-Rad, Richmond, VA, U.S.A) for 1 h. After blocking non-specific binding sites by incubation for 1 h at room temperature in 5% skimmed milk (dissolved in PBS-Tween 0.05%), the membranes were then incubated overnight at 4 °C with primary antibodies against GDNF, BDNF and  $\beta$ -actin, diluted 1:200, 1:200 and 1:300 respectively in blocking solution (The antibodies were specific anti-GDNF and anti-BDNF polyclonal rabbit immunoglobulins (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) or  $\beta$ -actin-specific mouse monoclonal antibody (CINVESTAV, Mexico). The following day the membranes were washed with PBS-Tween 0.05% and incubated for 1 h at room temperature with enzyme-labeled secondary antibodies (horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulins for BDNF and GDNF, and HRP-labeled goat anti-mouse immunoglobulins for  $\beta$ -actin, Zymed, CA, U.S.A.; used at dilutions of 1:2000 and 1:6000, respectively). After washing, the signals were developed with the Enhanced Chemiluminescence (ECL) system (Amersham Biosciences Europe, Freiburg, Germany).

The quantification of NTF expression was performed by digitizing the images in a BioDoc-It system (Bio-Rad, Richmond, VA, U.S.A), using the software application Lab Works 4.0 Image Acquisition for densitometric analysis. The expression of each factor was normalized against that of  $\beta$ -actin by calculating the NTF/ $\beta$ -actin optical density ratio for each electrophoretic run. Each sample was analyzed by triplicate.

#### Evaluation of the loss of dopaminergic cells

##### A. Tissue sampling

After concluding the *in vivo* studies, the rats received a higher dose of chloral hydrate (480 mg/kg body weight, i.p.) and were then perfused via the ascending aorta with 500 mL of 0.9% NaCl and 500 mL of a fixing solution containing 4% paraformaldehyde, 0.1% glutaraldehyde and 15% picric acid in 0.1 mol/L sodium phosphate, pH 7.4. The brains were extracted afterwards, placed in fixing solution for 1 h, washed with 0.1 mol/L sodium phosphate pH 7.4, cryoprotected in 7, 15 and 30% sucrose (24 h at each concentration), and frozen in liquid nitrogen. Coronal sections (20  $\mu$ m) were obtained from the areas corresponding to the SNc and mounted on microscopy slides previously coated with gelatin-chrome alum.

##### B. Immunohistochemical processing

In order to visualize the cells immunoreactive to tyrosine hydroxylase (TH), the slides with the histological sections were washed 3 times in PBS for 7 min each, followed by incubation during 20 min. in blocking and permeabilization solution (20% fetal bovine serum (FSB), 0.25% Triton X-100), and overnight coating at 4 °C with an anti-TH antibody (1:100 in 1% FSB/0.25% Triton X-100). The slides were then washed 3 times in PBS for 7 min each and incubated for 1 h with a biotinylated anti-mouse IgG antibody (1:500 in

1% SFB/0.25% Triton X-100). After washing again in PBS as described above, the slides were incubated for 1 h with a streptavidin-horseradish peroxidase conjugate, washed with PBS, and developed with H<sub>2</sub>O<sub>2</sub> as peroxidase substrate together with diaminobenzidine as the chromogen. The tissue sections were dehydrated using alcohol solutions of increasing concentrations, cleared with xylol and mounted in DPX.

The samples from animals where the 6-OHDA injection or the implantation of the cerebral microdialysis cannula had taken place at an anatomically incorrect location were excluded from the analysis.

**Data processing**

The normality of the data was verified with the Kolmogorov-Smirnov test in every case. The homogeneity of variance was checked by the Levene test.

The comparisons of escape latency, fall latency, average number of errors and extracellular concentrations of Glu and GABA at the PPN were done by one-way ANOVA, followed by Tukey’s test. In the case of the rotational activity induced by D-amphetamine the data did not follow a normal distribution, and therefore the comparison of this variable among the experimental groups was performed instead using a non-parametric analysis of variance (Kruskal-Wallis) followed by Mann-Whitney’s U-test.

A level of statistical signification of 0.05 was chosen for all analyses. The statistical software package Statistica CSS, version 6.1, was employed throughout.

**Results**

**Study of motor coordination by the transversal bridge test**

Escape latency in the 2.5 cm diameter, rectangular cross-section bridge was different only in the negative control groups (healthy rats or rats with false lesions)

vs. the groups with SNc lesions (whether pharmacologically treated or not) ( $p < 0.05$ ) (Figure 1A). Escape latency was not evaluated in bridges with smaller diameters (1 cm), since the rats with SNc lesions (pharmacologically treated or not) were unable to complete the time allotted for the tests.

When evaluating escape latency at the 2.5 cm diameter, circular cross-section bridge there was also a statistically significant increase for the groups with untreated ( $p < 0.01$ ) or treated ( $p < 0.05$ ) SNc lesions in comparison with the controls (Figure 1A). However, the increase in escape latency for the rats with SNc lesions treated with (-) nicotine was significantly smaller than for rats with untreated lesions, placing the former at an intermediate position between the negative controls and the untreated group (Figure 1A).

Although fall latency was quantified for almost all the experimental groups at the 2.5 cm diameter-rectangular cross-section bridge, it could not be measured for healthy rats and is not shown. No statistically significant differences were detected among any groups at the 2.5 cm diameter-circular cross-section bridge. However, the fall latency of rats with SNc lesions (treated or untreated with (-) nicotine) was significantly smaller ( $p < 0.001$ ) than that of the control groups at the 1 cm diameter bridges (Figure 1B).

**Quality of motor performance**

The direct observation of motor performance evidenced that the limbs contralateral to the lesion site in the rats receiving 6-OHDA but not treated with (-) nicotine had a small oscillation that swayed the body of the animal without achieving forward movement, and thus were not involved in forward locomotion. This group of rats never moved in a straight line along the bridges, instead alternating straight movements with tight turns that forced them to adopt increasin-

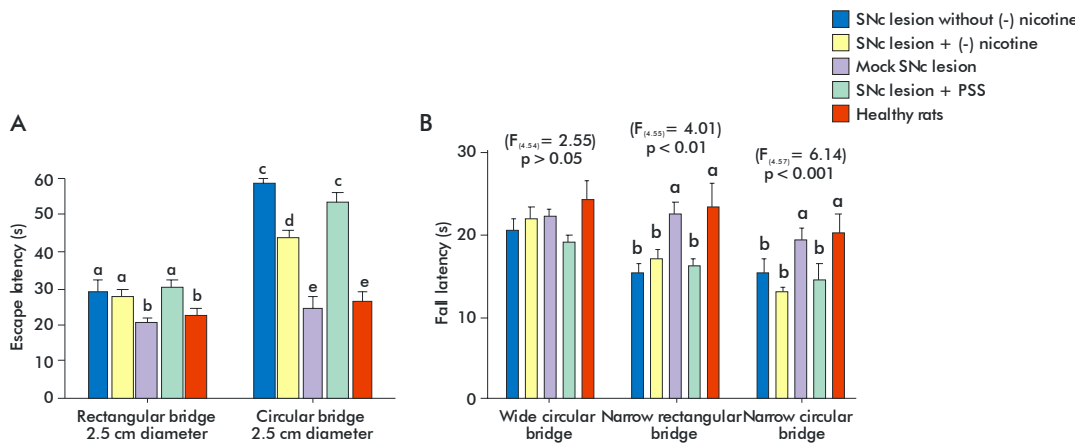


Figure 1. Effects of the systemic administration of (-) nicotine (1 mg/kg body weight, s.c.) in escape and fall latencies for the transversal bridge tests. A. Comparison of escape latencies for the 2.5 cm diameter rectangular ( $F_{(4,59)} = 5.13$ ,  $p < 0.05$ ) and circular ( $F_{(4,62)} = 9.22$   $p < 0.01$ ) cross-section bridges. The letters on top of each bar represent the differences between groups a vs. a b,  $p < 0.05$ , c vs. a d  $p < 0.05$ , c vs. a e,  $p < 0.01$ . B. Comparison of fall latency among the experimental groups, per bridge. The letters atop the bars represent the differences between the groups a vs. a b,  $p < 0.01$ . Experimental groups: rats with lesion of the *substantia nigra pars compacta* (SNc) without (-) nicotine treatment ( $n = 11$ ), rats with SNc lesion treated with (-) nicotine ( $n = 13$ ), rats with mock SNc lesions ( $n = 11$ ), rats with SNc lesions treated only with physiological saline solution (PSS) ( $n = 10$ ), healthy rats ( $n = 22$ ). The multiple comparisons were performed with a one-way ANOVA, followed by Tukey’s test. The bars represent  $\bar{X} \pm S.E.M.$



gly unstable positions as the diameter of the bridge was decreased (Figure 2A).

Although the rats with SNc lesions treated with (-) nicotine also turned spontaneously in tight circles, the hind limb contralateral to the SNc lesion site was less dysfunctional in their case, as evidenced by its use for support in a resting position (Figure 2B) and during locomotion (Figure 2C).

The rats with SNc lesions that were either untreated or treated with PSS instead of (-) nicotine had a significant ( $p < 0.05$ ) increase in the average number of errors during their performance at the 2.5 cm diameter rectangular cross-section bridge. In contrast, the rats with SNc lesions but treated with (-) nicotine had average numbers of errors undistinguishable from those of healthy individuals at the same bridge. When using circular cross-sectioned bridges, no statistically significant differences were found among the experimental groups for the 2.5 cm diameter bridge ( $p > 0.05$ ). However, all the groups with SNc lesions had a significant increase in the average number of errors ( $p < 0.05$ ) at the 1 cm bridge when compared to the control groups (healthy rats and rats with false SNc lesions) (Figure 3).

**Evaluation of the rotational activity induced by D-amphetamine**

The study of the rotational activity induced by D-amphetamine revealed a significant decrease ( $p < 0.01$ ) in rotational behavior for the rats treated by systemic administration of (-) nicotine in comparison with the individuals with SNc lesions but without pharmacological treatment. The attenuation of motor asymmetry in the treated group suggests, therefore, a positive effect for nicotine administration (Figure 4).

Evaluation of the effect of the systemic delivery of (-) nicotine on the extracellular concentration of Glu and GABA in the PPN and on the loss of dopaminergic cells at the SNc

**Study of the extracellular concentrations of Glu and GABA at the PPN**

There was a significant ( $p < 0.01$ ) decrease in the extracellular concentrations of both Glu and GABA at the PPN of the rats treated with (-) nicotine when compared to the individuals with SNc lesions that did not receive pharmacological treatment (Figure 5).

**Study of the loss of dopaminergic cells at the SNc**

The results of the immunohistochemical assays for tyrosine hydroxylase (TH) indicated that the pharmacological treatment with (-) nicotine protects SNc neurons from 6-OHDA-mediated neurotoxic injury. It was possible to observe TH+ cells at the medial and lateral regions of this structure (Figure 6A and B).

**Evaluation of the effect of the systemic delivery of (-) nicotine in the striatal expression of BDNF and GDNF**

The evaluation of the amounts of BDNF and GDNF by Western blotting yielded in both cases intense, well delimited bands after all electrophoretic runs (Figure 7A). The detection and quantitation of actin,



Figure 2. Most frequently observed errors during the execution of the transversal bridge test. A. Rat with a lesion in the substantia nigra pars compacta (SNc), adopting an unstable posture during spontaneous twisting. B and C. Rat with SNc lesion treated with (-) nicotine, using both hind limbs for support during resting (B) and locomotion (C) at the 2.5 cm diameter rectangular cross-section bridge.

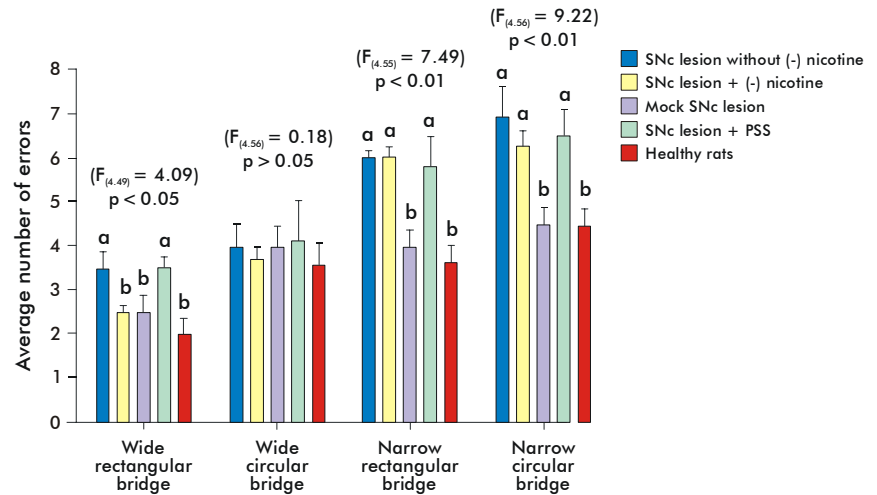


Figure 3. Comparison among the experimental groups for the average number of errors at the transversal bridge test. Different letters atop the bars represent statistically significant differences between the groups: a vs. b,  $p < 0.05$ . Experimental groups: rats with lesion of the substantia nigra pars compacta (SNc) without (-) nicotine treatment ( $n = 11$ ), rats with SNc lesion treated with (-) nicotine ( $n = 13$ ), rats with mock SNc lesions ( $n = 11$ ), rats with SNc lesions treated only with physiological saline solution (PSS) ( $n = 10$ ), healthy rats ( $n = 22$ ). The multiple comparisons were performed with a one-way ANOVA, followed by Tukey's test. The bars represent  $X \pm S.E.M.$

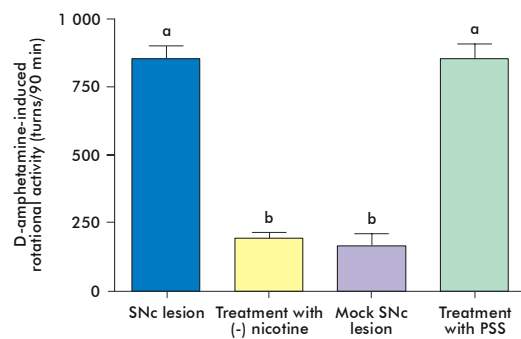


Figure 4. Comparison of the rotational activity induced by D-amphetamine (5 mg/kg body weight, i.p.) between rats with lesion in the substantia nigra compacta (SNc) ( $n = 10$ ), rats with SNc lesions treated with (-) nicotine ( $n = 18$ ), rats with mock SNc lesions ( $n = 10$ ) and rats with SNc lesions treated only with physiological saline solution (PSS) ( $n = 7$ ). Different letters atop the bars indicate statistically significant differences, a vs. a b,  $p < 0.001$ . The multiple comparisons were performed with a non-parametric ANOVA (Kruskal-Wallis test,  $H(3, 45) = 31.82$ ,  $p < 0.001$ ) followed by Mann-Whitney U-test. The error bars represent  $X \pm S.E.M.$

which is constitutively expressed in all cell types, allowed the normalization of the concentration of neurotrophic factors in reference to total protein expression levels in the St.

The data indicate that injuries to the SNc triggered by the administration of 6-OHDA in rats induce a significant increase ( $p < 0.01$ ) in the expression levels of both BDNF and GDNF at the St, as evidenced when compared to the homologous values in the healthy and false SNc lesion control groups (Figure 7B and C).

In the case of BDNF, the systemic administration of (-) nicotine before and 7, 14 or 21 days after the SNc lesion resulted in an increased striatal expression of this molecule (Figure 7B). On the other hand, the same treatment decreased the striatal expression of GDNF in hemiparkinsonian rats to levels undistinguishable from those of healthy controls (Figure 7C).

## Discussion

### Effect of the systemic administration of (-) nicotine on the motor disorders of hemiparkinsonian rats

The results indicate that the (-) nicotine systemic delivery scheme applied in this work promotes an improvement in motor performance for hemiparkinsonian rats. The treatment decreased the escape latency to values midway between those of healthy individuals and lesioned, untreated animals; additionally, it also decreased the average number of errors to the levels of the healthy control group at the 2.5 cm diameter rectangular cross-section bridge. Although no improvement was detectable when using bridges of smaller diameters (where fall latency and the average number of errors are the same as those observed for untreated hemiparkinsonian rats), the systemic administration of (-) nicotine also reduced motor asymmetry, as evidenced by the measurements of D-amphetamine-induced rotational activity.

A neuroprotective effect of (-) nicotine over the dopaminergic system has been proposed by several authors, based on the observed inverse relationship between addiction to this substance and the incidence of PD [19]. The delivery scheme used for (-) nicotine in this study is referred to in the literature as “subchronic administration”, and has been used frequently for the study of its neuroprotective effects in models of chelator-induced PD [20]. In our specific case, the administration of (-) nicotine immediately before the delivery of 6-OHDA followed two objectives: the early stimulation of the mechanisms promoting the release of NTF, and the use of the antioxidant properties of (-) nicotine for the attenuation of the effects of the neurotoxin [8, 21]. Although (-) nicotine is known to be quickly inactivated, a dose of 1 mg/kg body weight administered subcutaneously is able to cross the blood-brain barrier and reach the brain within 8 s, approximately. In these conditions, the intracerebral concentration of nicotine has been estimated to be around 5 nmol/L, at which the nicotinic receptors would be kept in a desensitized state for approximately 8 h [22]. The two follow-up doses, administered in this study every 30 min after the first administration, were used for maintaining and enhancing the initial effect of the

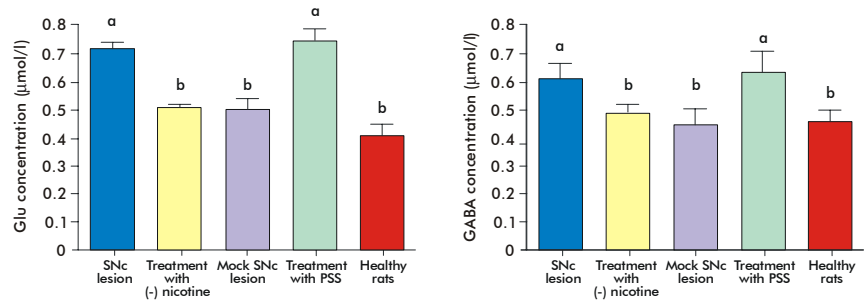


Figure 5. Effect of the systemic treatment with (-) nicotine (1 mg/kg body weight, s.c.) on the extracellular concentrations of glutamate (Glu) and  $\gamma$ -aminobutyric acid (GABA) at the pedunculopontine nucleus. A. Comparison of Glu concentration between the experimental groups ( $F(4, 39) = 15.45, p < 0.001$ ). B. Comparison of GABA concentration between the experimental groups ( $F(4, 36) = 2.96, p < 0.05$ ). Experimental groups: rats with lesions at the *substantia nigra compacta* (SNc) ( $n = 9$ ), rats with SNc lesions treated with (-) nicotine ( $n = 9$ ), rats with mock SNc lesions ( $n = 6$ ), rats with SNc lesions treated with physiological saline solution (PSS) ( $n = 6$ ) and healthy rats ( $n = 10$ ). The multiple comparisons were performed with a one-way ANOVA followed by Tukey's test. The letters atop the bars represent statistically significant differences: a vs. a,  $p < 0.01$ . The error bars represent  $X \pm S.E.M.$

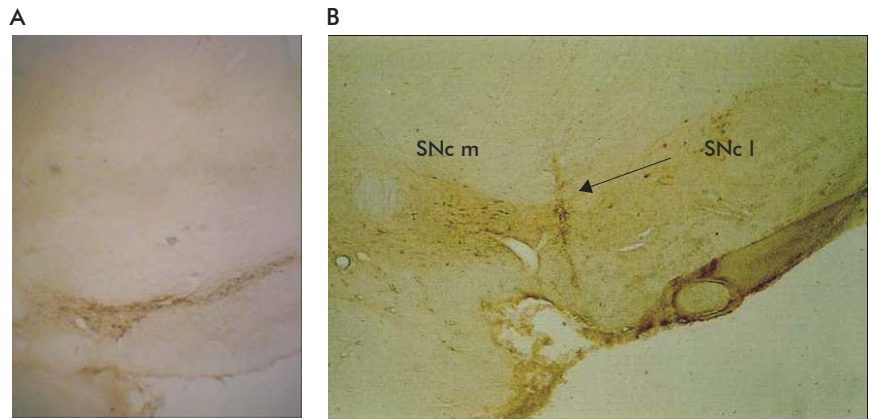


Figure 6. Microphotography of the immunodetection of tyrosine hydroxylase (TH) in a coronal section of the *substantia nigra compacta* (SNc) in a rat with a SNc lesion, treated by systemic administration of (-) nicotine. A. Microphotography (10X) of the right hemisphere, showing TH+ cells throughout the area corresponding to the SNc, at coordinate AP = -4.4 (according to Bregma). B. Local microphotography (20X) showing the cellular dopaminergic bodies of the medial and lateral SNc. The arrow indicates the trail left by the injection needle on the SNc area. SNcme and SNcla: Medial and lateral *substantia nigra compacta*, respectively.

drug. Similarly, the boosting doses administered 7, 14 and 21 days after the SNc lesion were intended to reinforce the effects of the exposition to nicotine [23, 24], by maintaining trophic support and promoting the release of striatal DA in order to counterbalance the dopaminergic deficiency associated to the injection of 6-OHDA [25-27].

During the study of motor activity by the transversal bridge test all the animals displayed the typical diagonally coupled alternating sequence that characterizes quadrupedal locomotion [28]. However, and in agreement with previous reports in the literature [29], the hemiparkinsonian rats were handicapped for the use of the limbs contralateral to the lesion site during both postural adjustments and ambulation. This dysfunction may be related to difficulties for adequately transferring the weight of the body and achieving an optimal postural adjustment in small supporting surfaces [30]. The observations presented here on the modifications of the locomotor pattern in hemipar-

19. Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue?. *Neurology* 1995;45:1041-51.

20. Maggio R, Riva M, Vaglini F, Fornai F, Molteni R, Armogida M, et al. Nicotine prevents experimental parkinsonism in rodents and induces striatal increase of neurotrophic factors. *J Neurochem* 1998; 71:2439-46.

21. Jenner P. Oxidative mechanisms in nigral cell death in Parkinson's disease. *Mov Disord* 1998;13:24-34.

22. Levin E. Nicotinic systems: an integrated approach. En: Levin ED, Ed. *Nicotinic receptors in the nervous system*. Londres, NY, Washington: Ed. Boca Raton, 2002:281-2.

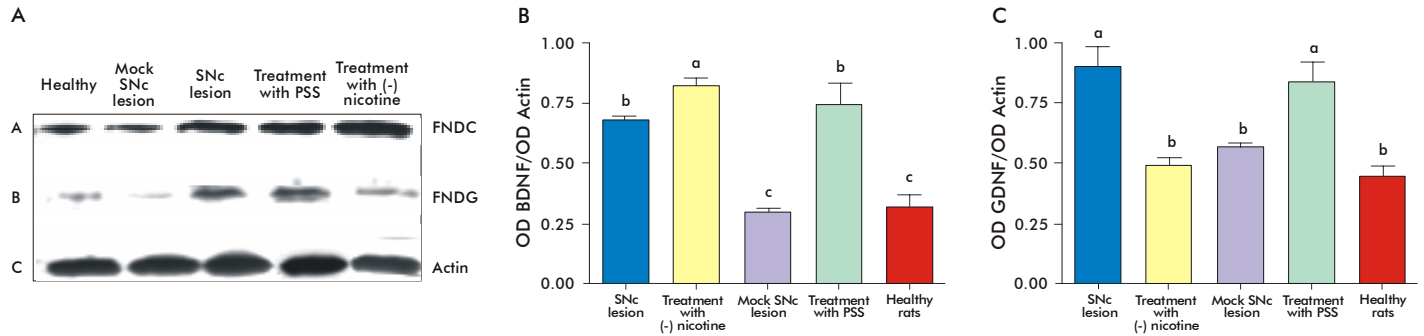


Figure 7. Comparison between the experimental groups for the striatal expression of neurotrophic factors, expressed as the optical density (OD) ratio between each factor and actin. A. Bands corresponding to the expression of brain-derived neurotrophic factor (BDNF), gliaderived neurotrophic factor (GDNF) and actin at the striatal tissue in each experimental group. B. Comparison of the striatal expression of BDNF ( $H(4, 18) = 13.27, p < 0.01$ ) between the groups. C. Comparison of the striatal expression of GDNF ( $H(4, 15) = 11.91, p < 0.01$ ) between the groups. Experimental groups: rats with untreated lesions of the *substantia nigra compacta* (SNc) ( $n = 4$ ), rats with SNc lesions treated with (-) nicotine ( $n = 4$ ), rats with mock SNc lesions ( $n = 3$ ), rats with SNc lesions treated only with physiological saline solution (PSS) ( $n = 4$ ), healthy rats ( $n = 4$ ). The comparisons were performed using a non-parametric ANOVA (Kruskal Wallis) followed by Mann-Whitney's U test. The letters atop the bars represent statistically significant differences: a vs. a b,  $p < 0.01$  in figure 7B, and a vs. a b,  $p < 0.05$ , b vs. a c,  $p < 0.01$ , a vs. a c,  $p < 0.001$  in figure 7C. The error bars represent  $X \pm S.E.M.$

kinsonian rats, dependent on the characteristics of the supporting surface, agree with those reported by other authors [31, 32].

It has been pointed out that striatal dopaminergic deficiencies represent an obstacle to the controlled application of the force demanded by the coordinated movement of all body parts. This obstacle may be manifested as a deficiency in the control of the affected limbs, as limb rigidity, as diminished motor reflexes or as a number of other sensory and motor deficiencies [33-35].

The treatment used in this study had a positive impact on motor performance during the tests with 2.5 cm diameter bridges. A milder loss of function in the hind limb contralateral to the 6-OHDA lesion site was observed in the hemiparkinsonian rats treated with (-) nicotine, and the time required to reach the escape platforms also decreased in this group when compared to untreated hemiparkinsonian individuals. This improvement is congruent with the similarities in the average number of errors between the group treated with (-) nicotine and the healthy controls. However, the limits of this recovery are shown by the results obtained when the determinants of support, equilibrium and locomotion -such as the shape and size of the supporting surface- approach critical values.

The treatment with (-) nicotine did not eliminate the tendency to spontaneously take tight turns in narrow circles, previously described for rats with SNc lesions. This behavior further pushes the center of mass off-balance, adding up to the difficulties already imposed by the diameter and the cross-sectional shape of the bridge [29]. The tendency to take tight turns further deteriorates the locomotor abilities of the animals and results in more frequent failures when attempting to hold onto the bridge or just staying on its surface [32].

As a whole, the data suggest that when cross-sectional shape and diameter are combined to implement high complexity situations, the systemic treatment with (-) nicotine applied in this study fails to improve the motor performance of the animals, which is dominated in this case by the locomotor dysfunctions associated with the lesions to the SNc

and is undistinguishable from that displayed by untreated hemiparkinsonian rats.

The lower motor asymmetry of the hemiparkinsonian rats treated with (-) nicotine may be related to smaller losses of dopaminergic cells at the SNc in these individuals. According to the literature, the nigral dopaminergic cells surviving a neurotoxic lesion can promote the release of DA from their storage vesicles when under the effects of a systemic administration of D-amphetamine [38, 39]. The net result of such a scenario would be a decrease in the number of amphetamine-induced turns and, therefore, the attenuation of motor asymmetry.

One of the mechanisms supposed to mediate the neuroprotective effect of (-) nicotine in PD is the activation of the presynaptic nicotinic cholinergic receptors found at intact nigrostriatal dopaminergic terminals [13]. It is known that this activation promotes DA release, and a small amount of this neurotransmitter is all that is needed to stimulate the striatal dopaminergic receptors [13, 40].

### Effect of the systemic delivery of (-) nicotine on the extracellular concentrations of Glu and GABA in the PPN

The restoration to normal levels of the extracellular concentrations of Glu and GABA in the PPN of hemiparkinsonian rats treated with (-) nicotine may be a manifestation of the general effects of the pharmacological treatment, which counterbalances any disequilibrium between the neurotransmitter systems of the two motor circuits at the BG. The PPN is a point of convergence for gabaergic and glutamatergic impulses originating at the "direct" and "indirect" pathways of the motor circuit [15]. Consequently, any functional alteration of these pathways will influence the release of neurotransmitters at the PPN and, therefore, their concentration in the extracellular space [41].

The improvement in dopaminergic activity afforded by the (-) nicotine treatment used in this study may originate from a decrease in the loss of dopaminergic bodies at the medial and lateral SNc. The surviving dopaminergic cells might exert some control over the St and counterbalance, to a degree, the disequilibrium

23. Brust JCM. Substances with neurotoxic potential: nicotine. En: Spencer P, Schaumburg H, Ludolph A, Eds. Experimental and clinical neurotoxicology. NY, Oxford: Oxford University Press Ed. 2000:860-2.

24. Rowell PJ. Effects of nicotine on dopaminergic neurotransmission. En: Levin ED, Ed. Nicotinic receptors in the nervous system. Londres, NY, Washington: Ed. Boca Raton, 2002:51-80.

25. Jones IW, Bolam PJ, Wonnacott S. Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. J Comp Neurol 2001;439:235-47.

26. Wonnacott S, Mogg A, Bradley A, Jones J. Presynaptic nicotinic acetylcholine receptors: subtypes mediating neurotransmitter release. En: Levin ED, Ed. Nicotinic receptors in the nervous system. Londres, NY, Washington: Ed. Boca Raton 2002:29-50.

27. Wonnacott S, Sidhura N, Balfour DJK. Nicotine: from molecular mechanisms to behavior. Curr Opin Pharmacol 2005;5:53-9.

28. Garcia Rill E. The PedunculoPontine Nucleus. Prog Neurobiol 1991;36:363-89.

29. Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS Plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 2000; 39:777-87.

30. Johnston RE, Schallert T, Becker JB. Akinesia and postural abnormality after unilateral dopamine depletion. Behav Brain Res 1999;104:189-96.

31. Tillerson JL, Castro SL, Zigmund MJ, Schallert T. Motor rehabilitation use in a unilateral 6-hydroxydopamine rat model of Parkinson's disease. Society Neurosciences Abstract 1998;25:672-78.

32. Miklyaeva EI, Martens DJ, Whishaw IQ. Impairments and compensatory adjustments in spontaneous movement after unilateral dopamine depletion in rats. Brain Res 1995;681:23-40.



between the pathways of the motor circuit. Nicotine inhibits the activity of the enzymes MAO-A and MAO-B, involved in the metabolism of DA [21]. This property would result in slower rates of enzymatic oxidation for DA and thus in reduced levels of oxidative stress at the nigral dopaminergic cells [21, 42]. The systemic administration of (-) nicotine may, therefore, prevent or attenuate this cascade of molecular events, ultimately resulting in reduced levels of cell death among the dopaminergic bodies of the SNc [43].

As mentioned above, the delivery scheme employed in this study was aimed at keeping the central nicotinic cholinergic receptors in a desensitized state (where desensitization is defined as a transition towards a stable, closed state of the receptor) [27]. According to the literature nicotinic receptors maintain, even after desensitization, a basal response to nicotine that results in the activation of protein phosphatases of the calcineurin family [44]. The proteins in this family act as high affinity sensors that activate even with very low intracellular calcium concentrations [44]. Under physiological conditions, an equilibrium is kept between protein kinases acting as low affinity sensors (requiring quantitatively superior calcium signals) and phosphatases. However, under conditions of desensitization of the nicotinic receptors, this balance is tipped towards the protein phosphatases, which then dephosphorylate a number of protein substrates, ultimately slowing down or completely aborting the triggering of mechanisms for cell death [44].

The inhibitory effect of (-) nicotine on the MAO-A and MAO-B enzymes, as well as the unbalanced equilibrium leading to the predominance of intracellular phosphatase activities, may contribute to the milder loss of dopaminergic cells from the SNc observed in this study for nicotine-treated hemiparkinsonian rats.

The effects of the partial restoration of the nigrostriatal dopaminergic activity are mediated by two main mechanisms: the correction of the increased striatal glutamatergic tone through the restoration of the DA-Glu interaction at the striatal level, and the restoration of dopaminergic stimulation of its striatal receptors [45]. Both mechanisms converge to correct the functional imbalance between the "direct" and "indirect" pathways of the motor circuit, and may partially explain the reestablishment of the normal extracellular concentrations of Glu and GABA at the PPN.

#### Effect of (-) nicotine treatment on the striatal expression of BDNF and GDNF in hemiparkinsonian rats

The results show that a neurotoxic injury to the SNc leads to an increase in the striatal expression of BDNF and GDNF. The (-) nicotine treatment schedule employed in this work further raised the expression levels of BDNF at the striatal tissue, but a similar effect was not observed for GDNF. One of the mechanisms hypothesized by other authors to explain the beneficial effects of the systemic delivery of (-) nicotine on parkinsonian symptoms is the increase in NTF expression levels [20, 46, 47].

Maggio *et al.* (1997) suggested that the neuroprotective effect of (-) nicotine in PD models might be based in the activation of mechanisms that modulate the susceptibility of dopaminergic neurons to excitotoxic damage [46].

The activation of the nicotinic cholinergic receptors of the dopaminergic cells of the SNc triggers an inward flow of Ca<sup>++</sup> to the neurons, which may result in the phosphorylation of a number of protein kinases, the transcription of early response genes and new protein synthesis, ultimately leading to enhanced synaptic plasticity and neuronal remodeling [47].

The data collected here about the behavior of BDNF agree with previous reports in the literature. Bustos *et al.* (2004) found an increase both in mRNA transcription and in BDNF expression levels at the St of hemiparkinsonian rats [48]. This finding has been linked to the activation of striatal N-methyl-D-aspartate (NMDA) glutamatergic receptors, based on the fact that BDNF controls the expression of different subunits of this receptor in cultured mesencephalic cells in a dose-dependent manner [49, 50]. In turn, Knott *et al.* (2002) showed increased immunoreactivity towards BDNF in association to the glial fibrillary acid protein in astroglia and ramified microglia during a post-mortem study of the St and SNc of parkinsonian subjects [51].

Neurotrophins such as BDNF can be synthesized by the glia. An increased activity of these molecules is suggestive of a glial response to signals originating from dopaminergic neurons exposed to an excitotoxic, oxidative, or other type of damage [51].

The available literature shows that the chronic and subchronic administration of (-) nicotine in rodents increases the expression of BDNF mRNA and the concentrations of BDNF itself [46]. The (-) nicotine administration scheme employed in our work is similar to the schemes employed by these authors.

The return to normality of the expression levels of GDNF at the St of hemiparkinsonian rats treated with (-) nicotine, as observed in this study, may be a typical result of the subchronic administration of this substance. Those publications reporting increased, rather than decreased, levels of GDNF have used chronic exposition schedules [52]. In any case, a neuroprotective effect for GDNF can not be discarded even at levels equivalent to those of healthy individuals, since Eslamboli *et al.* (2005) showed that the functional recovery of parkinsonian animals may be compromised if high levels of GDNF are expressed by means of a lentiviral vector [53].

As a whole, our results are consistent with a neuroprotective effect for the (-) nicotine treatment schedule employed in the present study, since it promotes changes in a model of hemiparkinsonism that lead to the adoption of support and locomotion strategies similar to those of healthy animals, as long as the diameter and cross-sectional shape of the supporting surface do not fall into critical values. The lower extracellular concentrations of Glu and GABA at the PPN may contribute in this model to a qualitative improvement in the transmission of motor signals that results in a better activation of muscular movement patterns. The increase in the amount of BDNF expressed at the St, together with milder losses of dopaminergic cells, can be part of the mechanisms behind this neuroprotective effect, attenuating the disequilibrium between the "direct" and "indirect" pathways of the motor circuits at the BG and, therefore, contributing to a detectable level of dopaminergic control over striatal activity.

33. Morgan M, Nomikos S, Huston JP. Behavioral analysis of asymmetries induced by unilateral 6-OHDA injections into the substantia nigra. *Behav Neural Biol* 1993;60:241-50.

34. Whishaw IQ, Castaneda E, Gorny BP. Dopamine and skilled limb use in the rat: more severe bilateral impairments follow *substantia nigra* than sensorimotor cortex 6-OHDA injection. *Behav Brain Res* 1992; 47:89-92.

35. Whishaw IQ, Gorny BP, Tran-Nguyen LTH, Castañeda E. Making two movements at once: impairments of movement, posture and their integration underlie the adult skilled reaching deficit on neonatally-depleted rats. *Behav Brain Res* 1994; 12:65-77.

36. Dani JA, Ji D, Zhou FM. Synaptic Plasticity and Nicotine Addiction. *Neuron* 2001;31: 349-52.

37. Corrigan WA, Coen KM, Zhang J, Adamson KL. GABA mechanisms in the pedunculopontine tegmental nucleus influence particular aspects of nicotine self-administration selectively in the rat. *Psychopharmacology* 2001;158:190-7.

38. Calne DB, Zigmond MJ. Compensatory Mechanisms in degenerative neurologic diseases. *Arch Neurol* 1991;48: 361-3.

39. Zigmond MJ, Hastings TG, Perez RG. Increased dopamine turnover after partial loss of dopaminergic neurons: compensation or toxicity? *Parkinsonism Relat Disord* 2002;8:389-93.

40. Smith ID, Sutton MA, Beninger RJ. Rotational bias in intact rats following intrastriatal injections of dopaminergic drugs. *Pharmacol Biochem Behav* 1997; 58(2):431-41.

41. Herrera-Marschitz M, You ZB, Gojny M, Silveira R, Godukhin OV, Chen Y, *et al.* On the origin of extracellular glutamate levels monitored in the basal ganglia of the rat by *in vivo* microdialysis. *J Neurochem* 1996;66:1726-35.

42. Blum D, Torch S, Lambeng N, Nissou NF, Benabid AL, Sadoul R, *et al.* Molecular pathway involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contributions to the apoptotic theory in Parkinson's disease. *Prog Neurobiol* 2001; 65:135-72.

43. Soto-Otero R, Méndez-Alvarez E, Hermida A, López AM, Labandeira JL. Effects of (-) nicotine and (-) conitine on 6-OHDA-induced oxidative stress and neurotoxicity: relevance for Parkinson's disease. *Biochem Pharmacol* 2002;64: 125-35.

44. Giniatullin R, Nistri A, Yakel J. Desensitization of nicotinic Ach receptors: shaping cholinergic signaling. *Trends Neurosci* 2005;28(7):371-8.

45. Chase Th, Justin D. Striatal dopamine- and glutamate-mediated dysregulation in experimental parkinsonism. *Trends Neurosci* 2000;23(10):86-90.

46. Maggio R, Riva M, Vaglini F, Fornai F, Racagni G, Corsini GU. Striatal increase of neurotrophic factors as a mechanism of nicotine protection in experimental parkinsonism. *J Neural Transm* 1997;104: 1113-23.



47. Belluardo N, Mudó G, Blum M, Fuxe K. Central nicotinic receptors, neurotrophic factors and neuroprotection. *Behav Brain Res* 2000;113:21-34.
48. Bustos G, Abarca J, Campusano J, Bustos V, Noriega V, Aliaga E. Functional interactions between somatodendritic dopamine release, glutamate receptors and brain-derived neurotrophic factor expression in mesencephalic structures of the brain. *Brain Res Brain Res Rev* 2004;47: 126-44.
49. Campusano J, Tapia-Arancibia L, Bustos G. The expression of NMDA receptors subunit I and metabotropic glutamate receptors subtypes 1 and 3, is differentially modulated by BDNF and glutamate receptors agonist in mesencephalic cultures. *J Neurochem* 2001; 78(1):18-33.
50. Taylor S, Srinivasan B, Wordinger R, Roque RS. Glutamate stimulates neurotrophin expression in cultured Müller cells. *Mol Brain Res* 2003;111:189-97.
51. Knott C, Stern G, Kingsbury A, Welcher AA, Wilkin GP. Elevated glial brain-derived neurotrophic factor in Parkinson's disease nigra. *Parkinsonism Relat Disord* 2002;8: 329-41.
52. Sun M, Kong L, Wang X, Lu X, Gao Q, Geller A. Comparison of the capability of GDNF, BDNF or both, to protect nigro-striatal neurons in a rat model of Parkinson's disease. *Brain Res* 2005;1052:119-29.
53. Eslamboli A, Georgievska B, Ridley R, Baker H, Muzyczka N, Burger C, *et al.* Continuous low-level glial cell line-derived neurotrophic factor delivery using recombinant adeno-associated viral vectors provides neuroprotection and induces behavioral recovery in a primate model of Parkinson's disease. *J Neurosci* 2005;25(4):769-77.

---

*Received in July, 2007. Accepted for publication in June, 2008.*