A nitric oxide synthase inhibitor decreases 6-hydroxydopamine effects on tyrosine hydroxylase and neuronal nitric oxide synthase in the rat nigrostriatal pathway

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ABSTRACT

There is evidence that nitric oxide plays a role in the neurotransmitter balance within the basal ganglia and in the pathology of Parkinson’s disease. In the present work we investigated in striatal 6-hydroxydopamine (6-OHDA) lesioned rats the effects of a nitric oxide synthase (NOS) inhibitor, NG-nitro-L-arginine (L-NOARG), given systemically on both the dopaminergic (DA) neuronal loss and the neuronal NOS cell density. We analyzed the DA neuronal loss through tyrosine hydroxylase immunohistochemistry (TH). The nitrergic system was evaluated using an antibody against the neuronal NOS (nNOS) isoform. Treatment with the L-NOARG significantly reduced 6-OHDA-induced dopaminergic damage in the dorsal striatum, ventral substantia nigra and lateral globus pallidus, but had no effects in the dorsal substantia nigra and in the cingulate cortex. Furthermore, L-NOARG reduced 6-OHDA-induced striatal increase, and substantia nigra compacta decrease, in the density of neuronal nitric oxide synthase positive cells. These results suggest that nitric oxide synthase inhibition may decrease the toxic effects of 6-OHDA on dopaminergic terminals and on dopaminergic cell bodies in sub-regions of the SN and on neuronal nitric oxide synthase cell density in the rat brain.

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Introduction

Parkinson’s disease (PD) is a major neurological disorder that affects movement, balance, and fine motor control. These impairments are related to the progressive degeneration of DA neurons in the substantia nigra pars compacta (SN), with a concomitant reduction of striatal dopamine levels (Agid, 1991). In addition to the striatum, the DA neurons from the SN are known to also innervate various extrastriatal structures, including the globus pallidus (GP) and the cortex, that are also...
affected in PD and in animal models of the disease (Solis et al., 2007; Anaya-Martinez et al., 2006; Debeir et al., 2005; Freeman et al., 2001; Gauthier et al., 1999; Prensa and Parent, 2001).

Although the etiology of PD is unknown, several lines of evidence suggest that oxidative stress may contribute to the mechanism of nerve cell death by the production of reactive oxygen species such as nitric oxide (Jenner, 2003).

Nitric oxide (NO), a free radical gas, is a highly reactive molecule (Dawson et al., 1994). It is produced from L-arginine and it acts as a neurotransmitter or neuromodulator when synthesized by the neuronal form of the enzyme NOS (Forstermann et al., 1991; Bredt and Snyder, 1990). nNOS is a constitutive, cytosolic, Ca2+/calmodulin-dependent enzyme (Bredt and Snyder, 1990; Klatt et al., 1992) that occurs in neuronal cell bodies, dendrites, and axons (Bredt et al., 1990).

Because NO has a short action time the inhibitors of NOS became a very effective way to study NO system function. Systemic application of L-arginine analogues such as L-NOARG (Dwyer et al., 1991) and N(G)-nitro-L-arginine methyl ester (L-NAME) has been shown to produce an in vivo (Carreau et al., 1994) and in vitro (Traystman et al., 1995) time-dependent irreversible inhibition of nNOS. Systemic and intrastriatal microinjection of selective (7-nitroindazole) and non-selective (L-NOARG and L-NAME) nNOS inhibitors in rodents interfere with motor behavior (Del Bel et al., 2002; De Oliveira et al., 1997; for review see Del Bel et al., 2005).

In experimental models of PD (reviewed by Kavya et al., 2006), immunoreactivity for nNOS in the SN is consistently increased 5 h after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment (Muramatsu et al., 2002), but decreased 1–7 days after injection. MPTP treatment also augmented the expression and activity of NO-activated guanylate cyclase (Chalimoniuk et al., 2004). We have previously found an increase in the nNOS cell density 35 days after 6-OHDA medial forebrain bundle-induced lesion (Gomes and Del Bel, 2003). Moreover, nNOS inhibitors, 7-NI and S-methyl thiocitrulline, exhibited protection in mice and baboons from MPTP-induced nigral cell loss (Schulz et al., 1995; Hantraye et al., 1996; Watanabe et al., 2007). Treatment with 7-NI was also reported to be protective against DA neuronal damage.

Fig. 1 – Effect of intrastriatal 6-OHDA and of the L-NOARG treatment on striatal and pallidal TH+ fibers. Photomicrographs show the sections at the anterior (1.3 mm), medial (0.2 mm) and caudal (−1.3 mm) striatum from Bregma of the sham (A–D), 6-OHDA (A′–D′) and 6-OHDA treated with L-NOARG (A″–D″). The 6-OHDA lesion caused a reduction in the TH+ fibers primarily in the middle and caudal part of the dorsal striatum (B′ and C′). The treatment with L-NOARG attenuated the loss of TH+ fibers (B″ and C″). Similar profiles of effects were observed in the GP (D–D″). Scale bar A–C″: 0.4 mm; D–D″: 0.06 mm.
induced by in vivo infusion of 1-methyl-4-phenylpiridinium (MPP+(+)) in freely moving rats (Di Matteo et al., 2006) and non-selective NOS inhibitors reduce amphetamine-induced rotations in a 6-OHDA model (Barthwal et al., 2001). Also Singh et al. (2005) found animals pretreated with a NOS inhibitor, L-NAME, exhibited complete protection against amphetamine-induced rotations in a 6-OHDA unilateral intrastriatal model. The authors concluded that augmented NO availability subsequent to inducible isoform of NOS (iNOS) induction play an important role in the initial phase of neurodegeneration. Furthermore, in nNOS knockout mice, MPP+ or MPTP-induced neuronal damage was less marked than in wild type mice (Przedborski et al., 1996).

However there are no studies concerning the influence of in vivo nNOS inhibition neither on the DA loss after intrastriatal 6-OHDA-induced lesion or in the subsequent modifications of nNOS. This neurotoxin, when injected into striatum, induces progressive and partial lesions of the nigrostriatal DA system in rat (Blandini et al., 2007; Winkler et al., 2002; Kirik et al., 1998; Sauer and Oertel, 1994) and mice (Pavon et al., 2006).

Thus, the aim of this work was to assess the effects of the NOS inhibition by L-NOARG given systemically on both the DA neuronal loss and the neuronal NOS cell density in rats after intrastriatal injections of 6-OHDA.

**Results**

First, the group of rats microinjected with saline (sham) and treated with L-NOARG did not show significant differences from sham rats treated with saline in this study.

**Effect of intrastriatal 6-OHDA injections on the density of TH+ fibers and cells and the L-NOARG treatment**

In the striatum: Representative cases from three different anatomical levels of striatum of the sham (A–C), 6-OHDA (A–C) and 6-OHDA treated with L-NOARG (A–C) rats are illustrated in the Fig. 1.

Densitometry revealed a significant reduction of TH+ in the caudal part of striatum of 6-OHDA rats in the lesioned side of the brain. This rats presented 22.7% of the optical density when compared to sham rats (F[2,14]=25.354; P<0.0001). More moderate, but still significant reduction was observed in the middle part of this structure (F[2,14]=14.227; P=0.001, Fig. 2), which optical density was 54.8% remaining. Anterior portion did not differ significantly from sham rats (F[2,14]=0.752; P=0.492 Fig. 2), presenting 94.2% optical density. We did not find significant differences in the contralateral striatum. Qualitative analysis indicated that the nucleus accumbens was not affected (results not shown).

Densitometry revealed a significant loss in the caudal striatum of the 6-OHDA treated with L-NOARG group, when compared with same side sham rats (70% optical density, F[2,14]=25.354; P<0.0001; Fig. 2). Reductions in the anterior and middle portions were not significantly different from sham (respectively 97.1% and 82.8% optical density). Regardless the 6-OHDA group, the reductions in the medial and caudal level were significantly smaller (respectively F[2,14]=14.227; P=0.001 and F[2,14]=25.354; P<0.0001, Fig. 2).

In the SN: Representative sections of SN from sham, 6-OHDA and 6-OHDA treated with L-NOARG rats are illustrated in the Fig. 3A, A’ and A” respectively.

The number of TH+ cell counts was significantly reduced in the ipsilateral SN of 6-OHDA (F[2,13]=9.134; P=0.005), with 50.7% remaining cells. The lesion induced significant loss of TH+ cells in the SNl (F[2,13]=7.832; P=0.008) and in the SN dorsal (F[2,13]=7.175; P=0.012). The loss of TH+ cells was greater in the SNv (46% present cells) and SNl (42%) than in the SN dorsal (67.4%, Fig. 3). Contralateral SN did not show significant modifications (results not shown).

The TH+ cell counts were significantly reduced in the ipsilateral SN of 6-OHDA treated with L-NOARG rats (Fig. 3B; F[2,13]=162.9.134; P=0.005), with 73.3% remaining cells. TH+ cell count 163

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Fig. 3 - (A) Effect of intrastriatal 6-OHDA injections on the TH+ neurons in the caudal substantia nigra (SN; −5.8 mm from Bregma). Photomicrographs show the SN in sham (A), 6-OHDA (A') and 6-OHDA treated with L-NOARG (A") (ipsilateral side). Scale bar: 0.3 mm. (B) Quantification of the TH+ neurons in the SN. Values represent the mean numbers (±SEM) of TH+ neurons within the ipsilateral SN and its dorsal (SNd), ventral (SNv) and lateral (SNI) portions. White columns represents sham group, black represents the 6-OHDA group and gray represents 6-OHDA treated with L-NOARG. Cell loss in the ventral and lateral SN was greater than in dorsal SN. L-NOARG treatment significantly reduced the TH+ cell loss in the ventral and lateral SN but not in the dorsal SN. * indicates significant difference from sham (effect of 6-OHDA). † indicates significant difference from 6-OHDA group (effect of l-NOARG). Number of rats per group=5 (P<0.05, one-way ANOVA followed by Duncan’s test).

In the striatum: Representative cases of ipsilateral striatum of 6-OHDA rats treated with saline or (B) with L-NOARG (D) are illustrated in Fig. 5. The mean density of nNOS+ neurons in the ipsilateral striatum of 6-OHDA rats was significantly increased when compared with sham group and this effect was modified by L-NOARG treatment (F[2,14] = 7.876; P=0.007).

The mean density increase of nNOS+ neurons in the ipsilateral striatum of 6-OHDA rats was significant at the anterior (+31%, F[2,13] = 6.768; P<0.05) and medial (+17.5%, F[2,14] = 7.876; P=0.006) levels when compared to sham. At the caudal portion, the mean density of nNOS+ cell did not differ significantly from sham rats. Fig. 2 shows the 6-OHDA-induced increase in the mean density of nNOS+ neurons and the L-NOARG modification of this effect along the striatum anteroposterior axis in 201 percentage from sham group.

The mean density of nNOS+ cells was not significantly increased in the anterior striatum of 6-OHDA rats treated with L-NOARG when compared to sham group. But, treatment with L-NOARG resulted in a reduction of nNOS+ cells, from +31% in the 6-OHDA rats treated with saline compared to +17.3% in 6-OHDA rats treated with L-NOARG. In the medial (−7.4%) and caudal levels (−14.1%), also, a significant decrease was found in the mean density of nNOS+ cells in the group 6-OHDA treated with L-NOARG when compared with 6-OHDA (respec- 211 tively: F[2,14]=5.453; P<0.05 and F[2,14]=6.356; P<0.05). We did not find significant differences in the striatum contralateral to microinjection (results not shown). Mean density of striatal 212 nNOS+ neurons: sham = 30 ± 1.7, 6-OHDA = 35 ± 2.0 and 6-OHDA 213 treated with L-NOARG = 27 ± 1.5.

In the SN: As the nNOS+ neurons could be found only in the dorsal tier of SN, we counted the number of nNOS positive neurons in this sub-region.
The mean density of nNOS+ cells was significantly reduced (47% remaining) in the ipsilateral SN of 6-OHDA ($F_{[2,12]}=3.787; P<0.06$) from the same side in sham rats. Contralateral SN did not present significant modifications (results not shown) after 6-OHDA.

The nNOS+ cell density was not significantly reduced in the ipsilateral SN of 6-OHDA treated with L-NOARG rats from sham group. Thus, as the 6-OHDA induced a significant decrease in the nNOS+ density of cells, treatment with L-NOARG resulted in an appreciable smaller loss of the density of nNOS+ cells in the SN (77.6% presenting in the 6-OHDA treated with L-NOARG X 53% in the 6-OHDA). Mean density of nNOS+ neurons total cell counts: 14.5 (±0.8) in the sham group, 7.7 (±0.8) in the 6-OHDA group and (11.2±2.0) in the 6-OHDA treated with L-NOARG group.

**Discussion**

Unilateral intrastriatal injections of 6-OHDA in rats resulted in a loss of TH+ fibers in the striatum and a retrograde loss of TH+ neurons in the SN, particularly in the ventral and lateral part. A concomitant decrease of TH+ fibers occurred in extrastriatal structures including the GP and the cingulate cortex. The pattern of TH+ fiber and cell loss in the protocol we used, which is considered a good model of progressive loss of DA-containing structures in PD (Kirik et al., 1998; Sauer and Oertel, 1994), was consistent with that reported by others (Przedborski et al., 1995; Winkler et al., 2002; Anaya-Martinez et al., 2006, Debeir et al., 2005). nNOS immunohistochemistry revealed an increase of nNOS cell density in the striatum and a decrease in the substantia nigra compacta after 6-OHDA.

Our results suggest a protective effect of NOS inhibition by L-NOARG. This effect can be due to activity of NO on dopamine transporter (DAT) and/or its biochemical interaction with the 6-OHDA.

It has been proposed that NO donors diminish the capacity of the DAT in a dose-, time- and temperature dependent manner (Pogun et al., 1994; Lonart and Johnson, 1994; Kiss et al., 2004). However, Volz and Schenk (2004) have reported that NO produced from L-arginine, a NOS substrate, affects DAT in a different manner to NO derived from NO donors. In that study, L-arginine increased DAT activity in the rat striatum by a NOS-dependent mechanism. Since 6-OHDA is a substrate for DAT (Blum et al., 2001), it is reasonable to propose that NOS inhibition would lead to less NO production, less DAT activity and hence could protect DA terminal from 6-OHDA access.

On the other hand, NO reacts with dopamine (Antunes et al., 2005) and 6-OHDA (Riobo et al., 2002; Palumbo et al., 2001) producing semiquinones, peroxinitrite and 6-hydroxydopamine quinone. These compounds are related to oxidative stress, which is recognized as an important mediator of neuronal death generation in the 6-OHDA model (Smith and Cass, 2006; Henze et al., 2005) and in PD (Jenner, 2003; Fahn and Cohen, 1992). In...
agreement, Hanrott et al. (2006) recently published that extra-
cellular auto-oxidation of 6-OHDA induces apoptosis in PC12
cells. Thus, an inhibition of NO production could offer some
protection from these events.

Our results are in agreement with previous studies show-
ing that NOS inhibition can protect against MPTP-induced
neurotoxicity in experimental animals (Schulz et al., 1995;
Hantraye et al., 1996; Watanabe et al., 2007).

The intrastratial 6-OHDA lesion induced a preferential loss
of TH+ neurons in the ventral SN as previously observed (Van
den Munchkof et al., 2006; Debeir et al., 2005). This ventral
cluster of cells provides a massive projection to the dorsal
striatum and lateral GP (Prensa and Parent, 2001; Debeir et al.,
2005). In addition, a lateral to medial gradient of TH+ loss in
the SN was described in PD (Hu et al., 2006; Hutchinson and Raff,
1999; Hutchinson et al., 2003) and in rat 6-OHDA model (Olds
et al., 2006; Fallon and Moore, 1978). Like the striatum and GP,
the pattern of DA degeneration observed in the cingulate
cortex can be associated with the pattern of neuronal loss in
the dorsal SN (Deutch et al., 1988; Haber and Fudge, 1997). Thus,
our results are consistent with previous anatomical reports
since the effects of the L-NOARG in some SN sub-regions were
reflected in their projection targets and collateralists.

One of the main findings of the present study is the L-NOARG
protective effect on the 6-OHDA induced modifications of nNOS
cell density, which was increased in the striatum and decreased
in the SN. The mechanisms by which deafferentation affects the
density of nNOS+ cells in the denervated circuitries is not fully
understood.

The interaction between nNOS and TH+ neurons and fibers
in the striatum is complex. NO has been proposed to regulate
the DA release and uptake (West et al., 2002; West and Galloway,
1998) and the increased nNOS cell density could result from a
compensatory augmentation in the activity of striatal nNOS+
terneurons, which are excited by an intact nigrostriatal
pathway through a D1-like receptor mechanism (Centonze
et al., 2002). Also, there are indications in the literature of an
increase in the nNOS cell density or NADPH-diaphorase activity
in the striatum after chronic deafferenation (Sancesario et al.,
2004; Morton et al., 1993; Gomes and Del Bel, 2003). Neuronde-
generation probably leads to rearrangements of the remaining
neurocircuitries, preserving and/or further impairing the
affected functions. Similar rearrangements may perhaps pro-
vide a background for functional alterations. NOS is required for
striatal plasticity events like long-term depression (LTD) of
cortico-striatal transmission, as well as metatropic glutamate
group I receptors, and both events seem to be linked by release
of endocannabinoids (Seryewa et al., 2007). LTD can be protec-
tive as glutamate release is enhanced at these synapses in PD, so
we can speculate that nNOS in the striatum will contribute to
the depression of cortical inputs and that the increase of nNOS
cell density could represent a compensatory mechanism.

Direct synaptic contact was demonstrated along the meso-
cortical and mesostriatal dopaminergic pathway either between DA
and NADPH-diaphorase (Fujiyama and Masuko, 1996) or NOS-
containing neurons (de Vente et al., 2000; Benavides-Piccione and
DeFelice, 2003) in the adult. In sections of the mesencephalon
from two-day-old pups, NOS-TH double-labeled neurons could
be seen in the SN, VTA, cingulate and frontoparietal cortex
(Gomez-Urquijo et al., 1999). However, such neurons became
infrequent in sections from one-month-old rats. Occasionally
some TH+ neurons in rat primary mesencephalic cultures
(Salum et al., in press). Thus, the reduction in nNOS+ neurons
in the substantia nigra compacta following nigrostriatal deaf-
erentation was probably due to a loss of mesencephalic neurons.

In conclusion, our results suggest that L-NOARG treatment
has a protective effect on nigrostriatal 6-OHDA-induced lesion.
It was consistent in terms of projection pathways of the DA
system and because it was able to reduce the lesion effects on
NO system. However, we can only speculate that events carried
d out by NOS inhibition and more studies are necessary to
elucidate the mechanism by which NOS inhibitors modify the
effects of 6-OHDA.

Experimental procedures

Animals

Adult male Wistar rats weighting 200–250 g were housed in
groups of five per cage in a temperature-controlled room
(23 °C), under 12 h light–dark cycle, with free access to food and
water. All experiments were performed in accordance with the
guidelines described in the European Communities Council
Directive of 24 November 1986 (86/609/EEC), and the experi-
mental protocol was carried out in compliance with the guide-
lines of the Brazilian Society of Neuroscience and Behavior for
the care and use of laboratory animals. Every effort was made
to keep the number of animals to a minimum and to minimize
suffering.

Drugs

Ascorbic acid (vitamin C, Sigma U.S.A.) and L-NOARG (Sigma)
were dissolved in saline. All drugs with exception to 6-OHDA
were administered intraperitoneally (i.p.), in a volume of 4 mL
kg. Doses were chosen based on previous studies (6-OHDA:
Gomes and Del Bel, 2003; L-NOARG: Marras et al., 1995; Del Bel
and Guimarães, 2000).

Surgical procedure

The animals were anesthetized with 2.5% 2,2,2-trimethoxymethanol
(10 mL/kg i.p., Aldrich) and fixed on a stereotaxic frame (David-
 Kopf) with the incisor bar 5 mm above the interaural line. A 370
striatal lesion was induced by four microinjections of 2 µL of the
6-OHDA (4×7 µg/2 µL of saline containing 0.02% ascorbic acid) 371
into the right striatum. The microinjections were made at four 372
locations within the striatum: 1) A: +1.3 L: 2.6 D: ~ 5.0; 2) A +0.4 L: 373
3.2 D: ~ 5.0; 3) A: ~ 0.4 L: 4.2 D: ~ 5.0; 4) A: ~1.3 L: 4.5 D: ~ 5.0 from
Bregma, according to the atlas of Paxinos and Watson (1998).

The microinjections were performed in a rate of 1 µL/min 374
with an infusion pump (Scientific, USA), and the needle was left 375
in place for an additional 180 s to prevent reflux. The movement 376
of an air bubble inside the PE-10 polyethylene tubing connecting
the micro-syringe with the needle confirmed drug flow. Sham-
operated animals were submitted to the same procedure but
received vehicle (saline containing 0.02% ascorbic acid) instead
of the neurotoxin.
Experimental groups

On day one animals received the first dose of either L-NOARG or saline (n = 5, i.p. dissolved in saline 4 ml/kg) or saline (n = 5, i.p.). On day two they were subjected to the stereotaxic surgical procedure to inject the 6-OHDA or saline into the striatum. Both L-NOARG and saline i.p. administrations were continued for three further days (total of 5 injections), once a day at 14:00 o’clock. The experimental groups were: saline/saline (sham, n = 6); saline treated with L-NOARG (n = 4); 6-OHDA/saline (named 6-OHDA, n = 5); 6-OHDA treated with L-NOARG (n = 5). Animals were sacrificed on day 36 after surgical procedure.

Histological analysis

Rats were sacrificed by terminal anesthesia (1.5 g/kg; 0.4 ml/kg urethane, i.p.) and perfused intracardially with 200 ml of 10 mM phosphate buffered saline, pH 7.4, followed immediately by 200 ml of freshly prepared 4% ice-cold paraformaldehyde in PBS. The brains were removed and placed in 4% paraformaldehyde in PBS for 2 h, cryoprotected in 30% sucrose in PBS, and then snap-frozen in isopentane (−40 °C cooled in dry ice) and stored at −70 °C until the immunohistochemical procedures were carried out.

Thirty five micrometer serial sections were cut within a cryostat (Leica). Neuroanatomical sites were identified using the Paxinos and Watson (1998) atlas. The anteroposterior (AP) localizations from Bregma of the analyzed areas were AP: 1.3, anterior striatum, AP: 0.2, medial striatum, AP: −1.3 mm, caudal striatum and AP: −5.8 mm, caudal SN (Fig. 6).

To ensure that neurons were not counted twice analysis was performed in every sixth section (i.e. separated by 210 µm). Adjacent brain sections were stained for TH, a marker for dopaminergic neurons, and for nNOS immunoreactivity.

Immunohistochemistry of TH and nNOS

TH immunohistochemistry was performed as previously described (Douhou et al., 2002). The same procedure was carried out for NOS.

Briefly, tissue sections were washed and then incubated 18–24 h with the primary antibody (TH: 1/1000, Peel Freez or nNOS: 1/1000, Emson — see Herbison et al., 1996). Sections were processed by the avidin-biotin immunoperoxidase method (Vectorstain ABC kit, Vector Lab) and immunopositive cells and terminals were visualized by addition of the chromogen 3,3’-diaminobenzidine (DAB; Sigma, 1 mg/ml) and hydrogen peroxide (0.2%).

Sections were mounted onto gelatin-coated glass slides, dehydrated in ethanol, cleared in xylene, and cover-slipped for microscopic observations. Immunopositive cells were revealed by a brown reaction product. Tissue from sham and lesioned groups was always processed at the same time.

In all experiments tissues from every group were always processed in the same assay.

Quantitative morphology

All the histological quantification was performed blindly. A preliminary qualitative analysis identified the regions with high neuronal labeling. Neurons which were positively stained exhibited labeled soma, dendrites and axons. The number of TH positive neurons in the SN and the number of nNOS positive neurons in the SN (Fig. 6) and striatum were counted, bilaterally, using a microscope (Nikon) equipped with a 60× objective (numerical aperture 1.4) and connected to an image analysis system (Mercator, Explora Nova, LaRochelle, France) equipped for stereological application. Results are expressed as the mean density of cells (number of positive neurons/0.5 mm² of the structure), calculated from results obtained in each brain side.

Labeling of TH positive fibers in the striatum was assessed by optical density measuring average pixel optical density over cell body and network. The optical density of a tissue area devoid of staining (corpus callosum) was measured (Mercator 450 Explora Nova computerized image analysis system), being the light intensity adjusted to give a background density value. The background was subtracted from all subsequent measurements. A mean value for staining intensity was calculated and expressed in arbitrary gray scale (relative optical density) units (ROD units; Morris et al., 1997). Results are expressed as a percentage of the same side in sham brains.

The counts and measurements were performed in 3–4 sections and then we calculate the mean value per rat.

Statistical analysis

All values are expressed as the mean ± SEM.

TH positive (TH+) and nNOS positive (nNOS+) cell counts were also as TH+ optical densities measurements from sham, lesioned and lesioned treated with L-NOARG rats were compared using one-way analysis of variance (ANOVA) followed by Duncan’s test, factor being treatment. A significance level of 0.05 was considered to be statistically significant. The degrees of freedom were designed as F. All statistical analyses were performed using SPSS for Windows (version 8.0).

Fig. 6 – Representation of the chosen distance from Bregma comprising the substantia nigra sub-regions for analysis: lateral (SNl), dorsal (SNd) and ventral (SNv) tiers. Extracted from Paxinos and Watson, 1998. The optical disector method was used to count TH+ and nNOS+ neurons in the SN at the caudal level of this structure (−5.8 mm from Bregma). We counted TH+ cells in the dorsal, ventral and lateral sub-regions of the SN. The boundaries of the SN subdivisions in the coronal plane were determined microscopically (4× objective) and marked on line drawings using a computer. As the nNOS+ neurons can be found only in the dorsal tier of SN, we counted the number of nNOS positive neurons in this sub-region.
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