Locus coeruleus and dorsal raphe neuron activity and response to acute antidepressant administration in a rat model of Parkinson’s disease

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Abstract
In addition to noradrenergic and serotonergic systems, dopaminergic neurotransmission seems to play an important role in the aetiopathogenesis of, and recovery from, depression. Moreover, the incidence of depression is higher in patients affected by diseases where the dopaminergic system is highly impaired, such as Parkinson’s disease. Here, we investigated the effects of dopamine degeneration on the activity and response to antidepressants of locus coeruleus (LC) noradrenergic and dorsal raphe nucleus (DRN) serotonergic neurons. To this end, single-unit extracellular recordings were performed in control and 6-hydroxydopamine (6-OHDA)-lesioned animals. In this latter group, LC neurons showed a lower basal firing rate as well as less sensitivity to the administration of the serotonin reuptake inhibitor, fluoxetine. The rest of electrophysiological parameters and the response to the administration of the $\alpha_2$-adrenoceptor agonist, clonidine and the noradrenaline reuptake inhibitor, reboxetine remained unaltered. In the DRN, dopamine depletion did not modify the basal electrophysiological characteristics and the response to clonidine or fluoxetine administration. In contrast, the administration of reboxetine more efficiently induced an inhibitory effect in the lesioned group. In additional analyses it was observed that while in control animals, LC and DRN basal firing rate was significantly correlated, this relationship was lost after the 6-OHDA lesion. In conclusion, dopaminergic degeneration alters LC neuron basal activity, the relationship/syntony between both nuclei, and their response to antidepressants. These findings shed fresh light on our understanding of the role of dopamine in depression and the mechanism action of antidepressants.

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Key words: $\alpha_2$-adrenoceptors, fluoxetine, noradrenergic neurons, reboxetine, serotonergic neurons.

Introduction

Despite progress made over the last 50 yr in understanding the function of neurotransmitter systems in mood disorders, we are still far from having a convincing account of the cause and treatment of depression. Most symptoms associated with depression are thought to arise from a noradrenaline and serotonin deficiency (Brunello et al. 2002). Moreover, there is considerable evidence linking the dopaminergic system with depression. Thus, lower concentrations of the dopamine metabolite, homovanillic acid, have been described in the cerebrospinal fluid, plasma, serum and urine of depressed patients (Engstrom et al. 1999; Hamner & Diamond, 1996; Lambert et al. 2000; Mitani et al. 2006). Further, there is a negative correlation between the levels of this metabolite and the severity of depression (Lambert et al. 2000). Imaging studies have shown that dopamine transporter binding is elevated in the basal ganglia of depressed patients (Brunswick et al. 2003). Clinically, the implication of dopamine in depression is evidenced by the elevated prevalence of this disorder in Parkinson’s disease (PD) patients. It affects 40–50% of patients, which is 3–4 times greater than that seen in the general population (Lieberman, 2006).
PD involves dysfunction or loss of dopamine neurons in the substantia nigra pars compacta that results in dopaminergic depletion in the striatum (Kish et al. 1988). Loss of dopaminergic neurons may be one of the mechanisms underlying depression in PD. Thus, an inverse correlation has been found between the severity of anxiety and depression symptoms and left anterior putamen dopamine transporter availability in PD patients (Weintraub et al. 2006); more extensive degeneration of dopaminergic neurons has been observed in the ventral mesencephalon of PD patients who are depressed than in those who are not (Willner, 1997). In addition, some authors have reported that l-dopa treatment alleviates depressive symptoms in some cases (Funkiewiez et al. 2003; Witt et al. 2006), but not in others (Choi et al. 2000; Cummings, 1992; Morrison et al. 2004).

The status of the dopaminergic system may influence not only the pathogenesis of depression, but also the response to antidepressant treatment. In fact, to improve the efficacy of antidepressant treatment, triple reuptake inhibitors (noradrenaline, serotonin, and dopamine) are currently being developed. In this line, the dopamine agonist pergolide improved depressed patients in an open, non-blind trial (Izumi et al. 2000). In addition, pramipexole, a $D_2/D_3$ receptor agonist, has been proven to be as effective as fluoxetine, a selective serotonin reuptake inhibitor (SSRI), in treating patients with severe depressive symptoms (Corrigan et al. 2000; Shannon et al. 1997) and to reduce the frequency of depression and anhedonia in PD patients with depression (Lemke et al. 2006).

Alterations in central noradrenergic and serotonin function have been implicated in the pathophysiology of affective disorders and most antidepressant agents target these two systems. The largest populations of central noradrenergic and serotonergic neurons are located in the locus coeruleus (LC) and dorsal raphe nucleus (DRN), respectively, and send afferents to all brain areas related to depression. Both nuclei undergo degeneration in PD (Braak et al. 2004). In addition, many serotonergic projections from the DRN degenerate, lower levels of serotonin are detected and 5-HT$_{1A}$ receptor and serotonin transporter binding are reduced (Doder et al. 2003; Guttman et al. 2007; Hornykiewicz, 1998). Regarding the noradrenergic system in PD, post-mortem studies have revealed substantial neuronal loss in the LC (Zarow et al. 2003), and a decrease in noradrenaline levels in the brain (Gesi et al. 2000; Hornykiewicz, 1998). Reduced levels of noradrenaline transporter have been reported in several noradrenergic areas of the brains of PD patients (Remy et al. 2005). It is still uncertain if these changes are simply due to dopaminergic neuron loss. The present study aims to shed light on our understanding of the role which dopamine plays in depression and in the response to antidepressant treatment. Thus, the influence of dopaminergic degeneration on basal activity and on the response to antidepressant drugs was evaluated in these nuclei. Hence, basal electrophysiological properties of LC and DRN neurons and their response to acute administration of reboxetine or fluoxetine, selective noradrenaline reuptake inhibitor (SNRI) or SSRI, respectively, were evaluated under dopamine-depleted conditions.

Materials and methods

Animals

Male Sprague–Dawley rats ($n=193$, weighing 150–175 g at the beginning of experiments) were used. Every effort was made to minimize the animals’ suffering and to use the minimum number of animals possible. Experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation at the University of the Basque Country. All the experiments were performed in compliance with the European Community Council Directive on ‘The Protection of Animals Used for Experimental and Other Scientific Purposes’ (86/609/EEC) and with the Spanish Law (RD 1201/2005) for the care and use of laboratory animals.

Experimental groups

Three groups of animals were used in this study: control, 6-hydroxydopamine (6-OHDA)-lesioned and sham animals. The sham group was made up of naive animals injected with apomorphine (0.5 mg/kg s.c.) and amphetamine (3 mg/kg, i.p.) and is hereafter referred to as the Apo+Amph group. This latter group was included in order to rule out any influence of the drugs used for the screening and to ensure the effect of the lesion.

Lesion surgery and rotational screening

6-OHDA lesions were performed as previously described (Bilbao et al. 2006). Animals were anaesthetized with chloral hydrate (400 mg/kg i.p.). Thirty minutes before lesion, desipramine (25 mg/kg i.p.) and pargyline (50 mg/kg i.p.) were administered. Four microlitres of 6-OHDA (2 µg/µl) was infused at a rate of 0.5 µl/min into the right medial forebrain bundle (relative to lambda; AP +4.4 mm, ML+1.2 mm, DV −8.2 mm).
To evaluate the extent of dopaminergic denervation, the turning behaviour was recorded 2 wk post-surgery over 60 min following apomorphine (0.5 mg/kg s.c.) and 90 min after d-amphetamine (3 mg/kg i.p.) administration. All animals included in the 6-OHDA-lesioned group showed more than five full-body turns/min (contralateral or ipsilateral, respectively).

Electrophysiological procedures

Single-unit extracellular recordings of LC and DRN neurons were performed as previously described (Martin-Ruiz & Ugedo, 2001; Migueléz et al. 2009). Animals were anaesthetised with chloral hydrate (400 mg/kg i.p.).

For LC recordings, the head was oriented at 15° to the horizontal plane (nose down). For DRN recordings, ligature and subsequent cutting of the sagittal sinus was needed. The recording electrode was lowered into the LC (relative to lambda: AP: -3.7 mm, ML: +1.1 mm, DV: -5.5 to -6.5 mm) or DRN (relative to lambda: AP: +1.0 mm, ML: 0 mm, DV: -4.5 to -6.0 mm). LC neurons were identified by standard criteria (Cedarbaum & Aghajanian, 1976) which included: spontaneous activity displaying a regular rhythm and a firing rate between 0.5–5 Hz; characteristic spikes with a long-lasting, positive-negative waveform, and a biphasic excitation–inhibition response to pressure applied to the contralateral hindpaw (paw pinch). Burst firing onset and end was defined as the concurrence of two spikes with interspike interval between 0.08 and 0.16 s, respectively (Grace & Bunney, 1984). DRN serotonergic neurons were identified by established criteria (Aghajanian et al. 1978), which include wide-duration action potentials between 1–2 ms, positive-negative spikes, a regular rhythm and a slow firing rate (0.5–3 Hz). Burst-firing DRN serotonergic neurons were identified as described previously (Hajos et al. 2007), by criteria including fired spike doublets or triplets with an intraburst time-interval <20 ms.

Firing patterns were analysed offline, using the computer software Spike2 (Cambridge Electronic Design, UK), and the following parameters were calculated: firing rate, coefficient of variation (percentage ratio of standard deviation to the mean interval value of an interspike time-interval histogram), percentage of spikes in burst, percentage of cells exhibiting burst firing, and response to intravenous drug administration. Only one cell was studied in each animal when any drug was administered.

The number of spontaneously active noradrenergic neurons was determined in nine additional tracks separated 50 μm around the first track through the LC prior to drug administration (10 tracks/rat).

At the end of each experiment, the brains were removed and stored in 20% sucrose solution. Coronal cryotome sections (50 μm) were cut through the striatum, substantia nigra, DRN or LC. All recording sites were located within the LC and DRN.

Immunohistochemistry

Sections containing the striatum, substantia nigra and LC were rinsed several times and processed free-floating. After inactivation of non-specific peroxidase reactions in 3% H2O2, sections were preincubated for 1 h in 5% normal goat serum. The primary antibody used for detection of tyrosine hydroxylase (TH; rabbit anti-TH) (AB 152, Chemicon International, USA) was diluted at 1:500 for 1 h. Both immunoreagents were diluted in phosphate-buffered saline containing 0.5% Triton X-100. The presence of the secondary antibody was revealed by incubating sections with the avidin–biotin–peroxidase complex (ABC kit, PK-6100, Vector Laboratories, USA), diluted 1:200 for 1 h. Both immunoreagents were diluted in phosphate-buffered saline containing 0.5% Triton X-100. The presence of the secondary antibody was revealed by incubating sections with the avidin–biotin–peroxidase complex (ABC kit, PK-6100, Vector Laboratories, USA) for 1 h. Finally, sections were stained with 3,3′-diaminobenzidine in the presence of 0.03% H2O2.

Three striatal sections for each animal (rostral, medial, and caudal levels) were optically digitized and the mean optical density (OD) associated with the striatum was calculated using NIH-produced image analysis software [Image] (http://rsb.info.nih.gov/ij/). The OD was expressed as a percentage of that of the contralateral intact side (100%) with the background associated with the cortex being set as 0%.

Stereological cell counting was performed in regularly spaced sections along the LC by the optical dissector method using the Mercator image analysis system (Explora Nova, France).

Drugs

Chloral hydrate, 6-OHDA, desipramine hydrochloride, pargyline hydrochloride, amphetamine sulphate, clonidine hydrochloride (Sigma-Aldrich, USA), reboxetine mesylate, RX-821002 hydrochloride (Tocris, UK), apomorphine hydrochloride (RBI, USA) and fluoxetine hydrochloride (Faes Farma, Spain). Chloral hydrate, desipramine, pargyline, clonidine and amphetamine were prepared in 0.9% saline; 6-OHDA and apomorphine, in distilled water containing 1 mg/ml ascorbate; and fluoxetine, RX-821002 and reboxetine in distilled water.
Table 1. Electrophysiological characteristics of locus coeruleus (LC) and dorsal raphe nucleus (DRN) cells recorded under basal conditions

<table>
<thead>
<tr>
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<th>LC noradrenergic neurons</th>
<th>DRN 5-HT neurons</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Lesioned</td>
</tr>
<tr>
<td>Active neurons per track</td>
<td>4.05 ± 0.48</td>
<td>2.92 ± 0.49</td>
</tr>
<tr>
<td>Basal firing rate (Hz)</td>
<td>2.04 ± 0.10</td>
<td>1.70 ± 0.08*</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>39 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>Neurons with burst firing (%)</td>
<td>54</td>
<td>60</td>
</tr>
</tbody>
</table>

Each cell was recorded for 180 s. The parameter ‘Active neurons per track’ was calculated in 10 consecutive tracks with eight animals in each group; * p < 0.01 vs. control group (Bonferroni’s post-hoc test).

Statistical analysis of data

Changes in firing rate are expressed as percentages of the baseline firing rate (mean firing rate during 3 min prior to drug injection). The response to drug administration was evaluated according to dose-concentration–effect curves nonlinearly fitted to a logistic three-parameter equation (Parker & Waud, 1971), and described previously (Miguelez et al. 2009). Statistical significance was assessed by means of two-way repeated-measures analysis of variance (ANOVA) or two-way non-repeated-measures ANOVA when some values were missing. For dose–effect curves with one independent variable, one-way ANOVA was used. All significant ANOVAs were followed by Bonferroni’s post-hoc test. For comparisons between two groups and for TH immunostaining quantification, the unpaired and paired Student’s t test were used, respectively. Finally, burst firing activity and frequency of neuronal response were analysed by non-parametric tests (Mann–Whitney) or χ², respectively. The level of significance was considered as p < 0.05.

Results

Basal electrophysiological activity in the LC in control and 6-OHDA-lesioned rats

A total of 305 noradrenergic neurons in the LC were recorded: 101 neurons from the control group (n = 34 animals), 124 neurons from the 6-OHDA-lesioned group (n = 24 animals) and 80 neurons from the Apo + Amp group (n = 20 animals). The number of spontaneously active cells was not significantly different between groups (Table 1). In contrast, the basal firing rate statistically differed between the groups (F₂,₃₀₈ = 4.18, p < 0.05, one-way ANOVA). Neurons recorded from the 6-OHDA-lesioned group showed a significantly lower basal firing rate compared to neurons from the control group (p < 0.05, Bonferroni’s post-hoc test) (Table 1). No significant differences were found in any of the other electrophysiological parameters (Table 1). Changes in firing rate may be attributed to dopaminergic degeneration, since the mean firing rate of neurons recorded from the Apo + Amp group was not different to that of the control group (p > 0.05, Bonferroni’s post-hoc test) (Table 1).

Effect of clonidine, fluoxetine and reboxetine on the firing activity of LC noradrenergic neurons in control and 6-OHDA-lesioned rats

To evaluate if the observed reduction in the basal firing rate of the LC was due to an increased sensitivity of α₂-adrenoceptors in the LC, we compared the effect of cumulative increasing doses (0.625–10 μg/kg i.v.) of the α₂-adrenoceptor agonist, clonidine, on LC neurons from control and 6-OHDA-lesioned rats. As shown in Fig. 1, dose–effect curves in both groups were not different (F₁,₄₄ = 1.44, p > 0.05 for lesion, repeated measures two-way ANOVA) (Fig. 1). The ED₅₀ values were also similar in the control and 6-OHDA-lesioned groups (2.24 ± 0.35 μg/kg, n = 6 and 2.93 ± 0.39 μg/kg, n = 6, respectively, p > 0.05, unpaired Student’s t test).

Then, we assessed if sensitivity to the reuptake inhibitor antidepressants, reboxetine and fluoxetine, was altered after dopamine degeneration. As previously reported by other authors (Szabo & Blier, 2001a; Wong et al. 2000), reboxetine administration (0.025–0.8 mg/kg i.v.) decreased the spontaneous firing activity of LC neurons in a dose-dependent manner. Following administration of the α₂-adrenoceptor antagonist RX 821002 (0.2 mg/kg i.v.) both groups recovered basal firing activity (Fig. 2a). Two-way repeated measures ANOVA revealed a slight difference in the dose–response curves from control and 6-OHDA-lesioned rats (F₁,₄₄ = 5.60 for the lesion) (Fig. 2b).
However, the ED$_{50}$ values from control (ED$_{50}$ = 0.11 ± 0.02 mg/kg, $n$ = 6) and 6-OHDA-lesioned rats (ED$_{50}$ = 0.08 ± 0.01 mg/kg, $n$ = 5) were not different ($p$ > 0.05, unpaired Student’s $t$ test).

As expected (Miguelez et al. 2009), fluoxetine (2.5–20 mg/kg i.v.) inhibited the firing activity of LC neurons in control ($n$ = 17), 6-OHDA-lesioned ($n$ = 7) and Apo + Amph ($n$ = 13) Animals. Fluoxetine produced a statistically significant weaker inhibition in the 6-OHDA-lesioned group ($F_{2,92} = 17.26, p < 0.001$ for lesion, two-way ANOVA). This difference was significant for fluoxetine at doses of 15 mg/kg and 20 mg/kg compared to the control group ($p < 0.05$ and $p < 0.01$, respectively, Bonferroni’s post-hoc test) (Fig. 3). These changes may be attributed to the lesion, since similar differences were observed for fluoxetine at doses of 10, 15, and 20 mg/kg between the 6-OHDA-lesioned group and the Apo + Amph group ($p < 0.05, p < 0.01$ and $p < 0.001$, respectively, Bonferroni’s post-hoc test) (Fig. 3). No differences were found in the LC neuron response to fluoxetine between the Apo + Amph group and the control group. The percentage of neurons that responded to fluoxetine was similar in all groups ($\chi^2 = 4.08, df = 2, p > 0.05$; 83%, 64% and 100% neurons in control, Apo + Amph and lesioned group, respectively). Neurons were considered to respond to fluoxetine if their firing rate was decreased by >15% from baseline after the highest dose.

**Basal electrophysiological activity in the DRN in control and 6-OHDA-lesioned rats**

In total, 418 serotonergic neurons were recorded in the DRN, 90 neurons in control ($n$ = 47 animals), 210

![Fig. 1. Effects of clonidine on locus coeruleus noradrenergic neurons in control and 6-OHDA-lesioned animals. Dose-effect curves for clonidine (0.625–10 µg/kg i.v.) in control and 6-OHDA-lesioned rats. Symbols represent mean ± S.E.M. of the percentage reduction from the basal firing rate ($n$ = 6 animals in each group).](http://ijnp.oxfordjournals.org/content/fig1)

![Fig. 2. Effects of reboxetine on locus coeruleus (LC) noradrenergic neurons in control and 6-OHDA-lesioned animals. (a) Representative example showing the effect of cumulative doses of reboxetine (0.025–0.8 mg/kg i.v.) in a control animal. Note that the subsequent injection of RX 821002 (0.2 mg/kg i.v.) completely reversed the reboxetine inhibitory effect. (b) Dose–effect curves illustrating the inhibitory effect of reboxetine on the LC neuron firing rate in control and 6-OHDA-lesioned rats. Symbols represent mean ± S.E.M. of the percentage reduction from the basal firing rate ($n$ = 9 and $n$ = 5 animals in the control and 6-OHDA-lesioned groups, respectively).](http://ijnp.oxfordjournals.org/content/fig2)
6-OHDA-lesioned (n = 8) and Apo+Amph (n = 5) animals. In all groups, subsequent injection of the 5-HT_{1A} receptor antagonist WAY-100635 (50–100 μg/kg i.v.) increased the firing rate to basal values (Fig. 4a). The 6-OHDA lesion caused a significant shift to the left of the dose–effect curve (F_{2,19} = 3.55, p < 0.0001 for the lesion, two-way ANOVA) (Fig. 4b) and decreased the ED_{50} value with respect to those of control and Apo+Amph groups (5.51 ± 1.23 mg/kg, n = 7; 10.75 ± 0.78 mg/kg, n = 7; and 11.63 ± 1.66 mg/kg, n = 5, for 6-OHDA-lesioned, control, and Apo+Amph groups, respectively; F_{2,16} = 7.84, p < 0.01, one-way ANOVA). These differences were observed between the 6-OHDA-lesioned and control or Apo+Amph groups (p < 0.05 and p < 0.01, respectively, Bonferroni’s post-hoc test). No differences were found between the Apo+Amph and control groups.

The inhibitory effect of reboxetine on DRN neurons has been related to an increment in terminal noradrenaline output and subsequent activation of inhibitory α_{2}-adrenoceptors. Thus, we examined changes in the sensitivity of these receptors using the α_{2}-adrenoceptor agonist, clonidine. As previously described (Haddjeri et al. 2004; Mongeau et al. 1993; Svensson et al. 1975), systemic administration of clonidine (0.625–40 μg/kg i.v.) to control (n = 9) and 6-OHDA-lesioned (n = 5) animals significantly depressed the spontaneous activity of DRN serotonergic neurons in a dose-dependent manner (Fig. 5). Two-way ANOVA revealed a slight difference in the dose–effect curves for clonidine between control and 6-OHDA-lesioned animals (F_{1,16} = 4.02, p < 0.05 for the lesion). However no significant differences were found in the ED_{50} values between the control and lesioned group (17.48 ± 2.59 μg/kg, n = 9 and 12.87 ± 5.58 μg/kg, n = 5, respectively; p > 0.05, unpaired Student’s t test).

In accord with other studies (e.g. Czachura & Rasmussen, 2000), fluoxetine (0.125–16 mg/kg i.v.) induced a decrease in the firing rate of DRN serotonergic neurons in a dose-dependent manner (Fig. 6a); posterior administration of WAY-100635 (50 μg/kg i.v., n = 13) restored the firing rate to basal values. In
6-OHDA-lesioned animals \((n=5)\), fluoxetine also inhibited DRN neuron activity. However, two-way ANOVA revealed no significant differences in the dose–effect curves obtained from control and 6-OHDA-lesioned rats \((F_{1,84}=1.77, p>0.05\) for the lesion\) (Fig. 6b) or in the corresponding ED\(_{50}\) values (control: \(1.10 \pm 0.21\) mg/kg, \(n=10\); 6-OHDA-lesioned group: \(1.43 \pm 0.37\) mg/kg, \(n=5\); \(p>0.05\), unpaired Student’s \(t\) test).

**Correlation between LC and DRN basal neuron activity in control and 6-OHDA-lesioned animals**

It is known that noradrenergic neurons modulate the serotonergic system (Baraban & Aghajanian, 1980; Bortolozzi & Artigas, 2003; Hertel *et al*. 1998) and vice versa (Guiard *et al*. 2008b; Haddjeri *et al*. 2004; Reader *et al*. 1986). In order to study the relationship between noradrenergic and serotonergic systems, we recorded neurons in the LC and DRN in the same animal and mean values obtained per animal in each nucleus were correlated. In controls \((n=6)\) and Apo + Amph \((n=8)\) animals, the basal firing rate of LC neurons was inversely correlated with the basal firing rate of DRN neurons \((r=-0.89, p<0.01\) and \(r=-0.94, p<0.0001\); Fig. 7a, b respectively). In contrast, in the 6-OHDA-lesioned group \((n=9)\), no correlation was observed between the parameters \((r=-0.38, p>0.05\), Fig. 7c).

**Effect of 6-OHDA lesion on striatal dopaminergic fibres and LC noradrenergic neurons**

All 6-OHDA-lesioned animals included in the study showed \(>95\%\) reduction (remaining dopaminergic fibres \(2.67 \pm 0.60\%\)) in TH-fibre density in the striatum on the side ipsilateral to the lesion (Fig. 8a, b). The precision of this method to quantify the degree of the lesion is confirmed by the linear relationship between the number of immunoreactive neurons in the substantia nigra pars compacta and the OD in the striatum (Bilbao *et al*. 2006). Additional quantification of TH-positive cells in the LC by stereological procedures did not show any difference between the lesioned and the control side in 6-OHDA-lesioned animals (intact side: \(7335 \pm 933.4\) neurons/mm\(^3\), \(n=8\); lesion side: \(7473 \pm 1050\) neurons/mm\(^3\), \(n=8\); \(p>0.05\), paired Student’s \(t\) test) (Fig. 8c–e).
Discussion

The present electrophysiological data show that striatonigral degeneration induces changes in basal neuron activity of the LC while leaving DRN neuron activity unaltered. Thus, a decrease in the firing rate of noradrenergic neurons was observed. In addition, these results also reveal that under control conditions there was a linear relationship between the firing rate values of LC and DRN neurons in the same animal but this correlation was not present after 6-OHDA lesion.

Moreover, neuron sensitivity to antidepressants was also altered after dopaminergic degeneration; fluoxetine was less effective on noradrenergic neurons, whereas reboxetine was more effective on serotonergic neurons. These findings are not due to changes in the sensitivity of \( \alpha_2 \)-adrenoceptors, since the response to the \( \alpha_2 \)-receptor agonist clonidine, was not modified.

In the LC, 6-OHDA lesion induced a decrease in the basal firing rate of noradrenergic neurons in the affected hemisphere without altering the number of active neurons or any other electrophysiological
parameter. To the best of our knowledge, there is only one publication which has analysed LC neuron electrophysiological properties in parkinsonian rats (Wang et al. 2009b). In that paper, an increment in LC firing rate and a reduction in the number of active neurons per track was reported in lesioned rats. Methodological variations may account for the apparent contradiction between that paper and the present study. In the Wang et al. study, the location of the toxin injection was hemilateral in the substantia nigra pars compacta. However, in the present study, the toxin was injected into the medial forebrain bundle and the noradrenaline uptake site inhibitor, desipramine, was administered prior to the lesion which results in a substantial preservation of dopaminergic cells in the ventral tegmental area (Andersson et al. 1999) and no damage of noradrenergic neurons in the LC. Here, we have confirmed that the dopaminergic lesion did not reduce the number of neurons in the LC using both electrophysiological and stereological methods. Thus, the observed changes in LC activity may be attributed to dopaminergic denervation. It is well established that LC activity is mainly regulated by somatodendritic \( \alpha_2 \)-adrenoceptors which exert a tonic inhibitory control (Cedarbaum & Aghajanian, 1976; Svensson et al. 1975). In this line, some studies show that dopamine produces an inhibitory effect on LC neurons through activation of \( \alpha_2 \)-adrenoceptors (Guiard et al. 2008a). However, changes in these receptors are not necessarily responsible for the reduced firing rate, since neurons from lesioned and control groups responded in a similar way to the systemic administration of clonidine. Similarly, we did not find significant changes in the response to reboxetine which is due to an increment in the level of noradrenaline in the synaptic cleft and a subsequent interaction with \( \alpha_2 \)-adrenoceptors (Szabo & Blier, 2001a). Under control conditions, substantial amounts of dopamine are present in the LC (Kaehler et al. 1999a, b; Lin et al. 2008), where it is used as the precursor of noradrenaline or as a neurotransmitter itself. Thus, dopamine and the dopaminergic agonist (+)-3-PPP inhibit electrical activity of LC noradrenergic neurons (Cedarbaum & Aghajanian, 1977; Elam et al. 1986; Guiard et al. 2008a) whereas the dopaminergic antagonist, haloperidol, enhances it (Nilsson et al. 2005). However, no effects have been found with the dopaminergic agonists quinpirole, pramipexole or apomorphine (Cedarbaum & Aghajanian, 1977; Chernoloz et al. 2009; Guiard et al. 2008b). On the other hand, dopamine may also stimulate the LC acting on \( \alpha_2 \)-adrenoceptors (Lin et al. 2008). In our study, the dopaminergic damage induced a decrease of LC basal neuron firing. Thus, it is suggested that dopaminergic degeneration may compromise the equilibrium between the dopamine activation of receptors involved in LC inhibition or stimulation.

Our data show that the response of LC neurons to fluoxetine administration is diminished after dopaminergic degeneration. These results are in accord with those of Chenu et al. (2007) who reported that after dopamine degeneration induced by intracerebroventricular 6-OHDA infusion, the efficacy of the SSRIs citalopram and paroxetine in the forced swimming test was abolished, while the effect of desipramine and imipramine remained unchanged. 6-OHDA injection into the DRN attenuates the anti-immobility effects of fluoxetine in the tail suspension test (Cryan et al. 2004). Recently, we have shown that fluoxetine inhibits LC neurons in vivo through a mechanism involving noradrenaline interacting with \( \alpha_2 \)-adrenoceptors (Miguelez et al. 2009); however, other receptors, such as 5-HT\(_{1C}\) or 5-HT\(_{2A}\) receptors are also known to mediate these effects (Bymaster et al. 2002, Szabo & Blier, 2001b). In 6-OHDA-lesioned animals, down-regulation of striatal 5-HT\(_{1C}\) receptor mRNA has been reported (Numan et al. 1995; Zhang et al. 2007b). While up-regulation of mRNA (Zhang et al. 2007b) but decrease of binding (Li et al. 2009) in the striatal 5-HT\(_{2A}\) receptor have been described in 6-OHDA-lesioned animals. So far, there is no publication that had studied those receptors in the LC under dopamine-depleted conditions. However, if similar changes on 5-HT\(_{1A}\) and/or 5-HT\(_{2C}\) expression also take place on the LC, the down-regulation of 5-HT\(_{1C}\) receptors could lead to the observed weaker inhibition of the LC.

To date, few studies on the electrophysiological characteristics of DRN serotonergic neurons in parkinsonian animals have been published and no consensus has emerged from them. Thus, some authors have reported an increase (Kaya et al. 2008; Wang et al. 2009a; Zhang et al. 2007a), while others have observed a decrease (Guiard et al. 2008b) in the firing rate of DRN neurons in parkinsonian rats under basal conditions. These discrepancies, reminiscent of those found with LC neurons in parkinsonian rats, could also be attributed to methodological factors. However, in some cases, opposite results were obtained using the same 6-OHDA-lesioned rodent model (Guiard et al. 2008b; Kaya et al. 2008). It is important to remember that in animal models of PD, striatal serotonin levels have been reported to be increased (Balcicglu et al. 2003; Commins et al. 1989; Karstaedt et al. 1994; Zhou et al. 1991) unchanged (Breese et al. 1984; Carta et al. 2006) or decreased (Aguiar et al. 2006, 2008). These findings indicate that these changes in the serotonergic
system may be highly dependent on the location and extension of dopaminergic degeneration. In this line, behavioural experimental findings are also inconsistent. Thus, manipulation of dopaminergic transmission in the pathway between the ventral tegmental area and the nucleus accumbens has an antidepressant-like effect in rats (Espejo & Minano, 1999; Renard et al. 2001). 6-OHDA lesions in the substantia nigra and ventral tegmental area induce depressive-like behaviour (Winter et al. 2007). In contrast, intracerebroventricular infusion of 6-OHDA does not cause altered depressive-like behaviour (Chenu et al. 2007).

Activation of \( \alpha_2 \)-adrenoceptors on the cell body and terminals of noradrenergic neurons decreases the release of noradrenaline and the subsequent excitatory input to \( \alpha_2 \)-adrenoceptors in the DRN (Haddjeri et al. 2004; Mongeau et al. 1993; Svensson et al. 1975). After dopaminergic degeneration, the sensitivity of DRN neurons to reboxetine increases. Changes in the \( \alpha_2 \)-adrenoceptor, which mediates the inhibition of 5-HT neurons, may not account for these changes, since sensitivity to clonidine was unaltered. Linner et al. (2004), showed that low doses of reboxetine increase the firing rate of DRN neurons by a mechanism that may involve \( \alpha_2 \)-adrenoceptors while higher doses have an inhibitory effect on the DRN. This latter effect has been attributed to the blockade of serotonin transporters (Millan et al. 2001; Wong et al. 2000) which enhances extracellular levels of serotonin that act on inhibitory 5-HT\(_1A\) autoreceptors. Indeed in the present study and in Linner et al. (2004), the effect of reboxetine on DRN was reversed by the 5-HT\(_1A\) receptor antagonist supporting the involvement of 5-HT\(_1A\) receptor in the effect of high doses of reboxetine. However, fluoxetine induced similar inhibitions in control and 6-OHDA-lesioned animals suggesting that 5-HT\(_1A\) receptors and the serotonin transporter are not affected after dopaminergic damage. As mentioned above, \( \alpha_2 \)-adrenoceptors are involved in the effect observed with low doses of reboxetine, which could influence the response when high doses are administered. However, the exact mechanism by which reboxetine has a higher effect on DRN neurons after dopaminergic damage remains unclear.

Reciprocal projections between the LC and DRN have been reported (Kim et al. 2004). Pharmacological studies have demonstrated that the firing activity of serotonergic neurons in the DRN depends on a tonic noradrenergic activation (Baraban & Aghajanian, 1980; Fritschy & Grzanna, 1990; Haddjeri et al. 2004). On the other hand, it has also been reported that selective lesions of the serotonergic system increase TH activity in the LC and the firing rate of noradrenergic neurons (Chamba et al. 1991; Crespi et al. 1980), while noradrenergic neuron lesion was found to be associated with low rate discharge of raphe neurons (Svensson et al. 1975). The present work shows, for the first time, that under control conditions there is a good correlation between the firing rate of LC and DRN spontaneously active neurons, recorded in the same animal. However, this correlation is broken in 6-OHDA-lesioned animals, since the lesion induced changes in the basal firing rate of LC neurons, but not in that of DRN neurons.

In PD patients with depression, reboxetine and fluoxetine are often treatments of choice for treating the depressive symptoms. SSRIs have been widely used, but the results are not always satisfactory since no efficacy, worsening PD or high incidence of side-effects have been reported (Leentjens et al. 2003; van de Vijver et al. 2002; Weintraub et al. 2006). Our results would suggest that cases in which a lack of effect is observed may be due to the weaker effect of fluoxetine on the noradrenergic system in parkinsonian conditions. Reboxetine has been proven to be effective and well tolerated in depressed PD patients (Leinke, 2002; Pintor et al. 2006). Moreover, the dose used in PD patients is lower than that used to treat major depression (Langworth et al. 2006; Papakostas et al. 2008). In our study, we have observed that reboxetine is able to induce a higher inhibition of DRN neurons in parkinsonian animals, whereas fluoxetine has less effect on LC neurons. Two recent clinical studies comparing the efficacy of desipramine with citalopram and nortriptyline with paroxetine in patients with PD and depression have reported that the SNRIs were more efficient than the SSRIs in alleviating depression symptomatology (Devos et al. 2008; Menza et al. 2009).

In conclusion, these data show that the degeneration of the nigrostriatal pathway is related to a lower basal firing rate of LC noradrenergic neurons, less sensitivity of LC neurons to SSRI antidepressants and a higher effect of SNRIs on DRN serotonergic neurons. These findings provide an electrophysiological basis for understanding changes in the effectiveness of antidepressants which have been clinically observed in depressed PD patients.

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Statement of Interest

None.

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