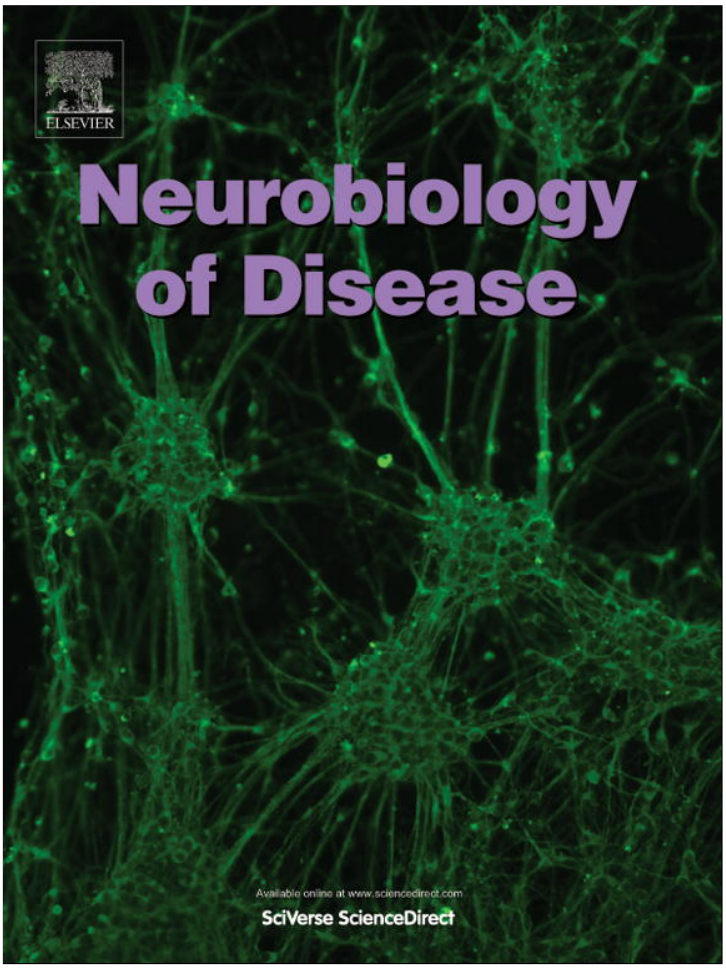

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Activation of subthalamic alpha 2 noradrenergic receptors induces motor deficits as a consequence of neuronal burst firing

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ABSTRACT

The subthalamic nucleus (STN) plays a key role in the pathophysiology of Parkinson's disease. This was demonstrated by the fact that STN neurons express more bursts in animal models of the disease and by the ability of STN inactivation to alleviate motor deficits. However, the origin of the bursts and the causal link between STN bursts and motor deficits remain unknown. The present study aimed to investigate the role of noradrenergic receptor modulation on the firing activity of STN neurons and the impact of this modulation on locomotor activity in sham and 6-hydroxydopamine-lesioned rats. Using selective agonists and antagonists of α1- and α2-adrenergic receptors (AR), we show that local infusion of clonidine, an α2-AR agonist, induced a switch from tonic to bursty pattern without changing the firing rate. This change in the pattern was prevented by the local infusion of idazoxan, an α2-AR antagonist. Furthermore, clonidine injection into the STN reduced locomotor activity in sham and 6-hydroxydopamine-lesioned rats. In contrast, local injection of phenylephrine, an α1-AR agonist, increased the firing rate of STN neurons without changing the firing pattern. In parallel, phenylephrine did not change locomotor activity. This is the first evidence showing the implication of α1-ARs in the modulation of firing rate and α2-ARs in the modulation of the firing pattern of STN neurons. Furthermore, our data provide also evidence that activation of the STN α2-ARs plays a key role in the genesis of subthalamic burst activity, which may be at the origin of motor deficits.

Introduction

Parkinson's disease (PD) is a neurological disorder characterized by the manifestation of motor symptoms such as akinesia, rigidity and rest tremor. Despite the focus on dopamine (DA) cell degeneration in the substantia nigra pars compacta (SNc) (Ehringer and Hornykiewicz, 1960), PD is characterized also by a dysfunction of the noradrenergic neurotransmission (Bertrand et al., 1997; Fornai et al., 2007). Several studies have shown that blockade of α2-adrenergic receptors (α2-ARs), mainly inhibitory autoreceptors (Langer, 1974; Starke, 1972), reversed tremor and rigidity induced by reserpine in the rat (Colpaert, 1987) and potentiated ipsilateral circling induced by the DA releasing agent amphetamine in the unilateral 6-hydroxydopamine (6-OHDA)-lesioned animals (Chopin et al., 1999; Mavridis et al., 1991). α2-AR antagonists also improved parkinsonian motor disabilities in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Bezard et al., 1999; Colpaert, 1987) and preserved the antiparkinsonian effects of L-3,4-dihydroxyphenylalanine (L-DOPA) while decreasing L-DOPA-induced dyskinesia (Gomez-Mancilla and Bedard, 1993). Furthermore, we have recently shown that systemic administration of α1- and α2-AR agents was able to modulate locomotor activity in the rat and that behavioral changes were paralleled by changes in the neuronal activity of subthalamic nucleus (STN) (Belujon et al., 2007). This nucleus has been shown to play a key role in the pathophysiology of PD. This was demonstrated by the fact that, in rodent and primate models of PD, STN neurons are more bursty than in normal animals (Bergman et al., 1994; Hassani et al., 1996; Ni et al., 2001; Parr-Brownlie et al., 2007) and by the ability of STN ablation (Bergman et al., 1990; Guridi and Obeso, 2001) and high frequency stimulation (Benabid et al., 2000; Benazzouz et al., 1993) to alleviate PD motor symptoms. Furthermore, anatomical studies revealed that the STN receives NA afferents from the LC (Boyajian et al., 1987; Canteras et al., 1990; Parent and Hazrati, 1995; Wang et al., 1996), which are relatively sparse (Cragg et al., 2004; Swanson and Hartman, 1975). Although previous in vitro electrophysiological studies on slices (Arcos et al., 2003; Belujon et al., 2007) have shown that STN neurons have functional α1- and α2-ARs, the impact of these receptors on STN neuronal activity and the motor behavior remain unknown. Thus, the present study aimed to investigate the effects of local injection of selective

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agonists and antagonists of α1- and α2-ARs upon (i) the firing rate and patterns of extracellularly recorded STN neurons and (ii) on the locomotor activity. These electrophysiological and behavioral studies were carried out in sham and 6-OHDA-lesioned rats.

Materials and methods

Animals

Adult male Sprague Dawley rats, weighing 280–380 g, were used for behavioral studies and in vivo electrophysiological experiments. Animals were provided by the "Centre d'Elevage Depré" (Saint Doulchard, France) and arrived at least 1 week before use. They were housed four per cage under artificial conditions of light (light/dark cycle; lights on at 7:00 A.M.), temperature (24 °C), and humidity (45%) with food and water available ad libitum. All animal experiments were carried out in accordance with European Communities Council Directive 2010/63/UE. The study received approval from the local Ethics Committee (Bordeaux, France).

Guide cannula implantation

Two weeks after 6-OHDA injection rats were placed in a stereotaxic frame (Unimecanique, Paris, France) under xylazine/ketamine anesthesia (ketamine hydrochloride (75 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p), Sigma). Each animal was implanted with a guide cannula with the tip 1 mm above the STN at coordinates 3.8 mm posterior to bregma, 2.5 mm lateral to the midline, and 7 mm below the skull according to the brain atlas of Paxinos and Watson (1996). The guide cannula was attached to the skull with stainless steel screws and dental cement. An obturator inside the guide cannula was then placed to prevent obstruction. Rats were placed in the animal facilities for one week to recover.

Drugs

Drugs were chosen on the basis of their different affinity for their preferential receptors. Phenylephrine was chosen as α1-AR agonist and benoxathian as α1-AR antagonist. In a similar manner, clonidine and idazoxan, α2-AR agonist and antagonist respectively, were selected. All drugs were purchased from Sigma (Saint-Quentin Fallavier, France). A dose of 20 μg/μl was used for all the drugs except for benoxathian (10 μg/μl). These doses were chosen on the basis of a detailed literature search, showing a significant behavioral effect in the rat (Crestani et al., 2008). The drugs were dissolved in 0.9% sterile saline and infused slowly into the STN via a digital microsyringe (Hamilton Company, Nevada, USA). In 6-OHDA-lesioned animals, as the toxin was injected unilaterally, NAergic agents were also injected unilaterally in the same 6-OHDA-lesioned hemisphere.

6-OHDA injection

As described previously (Delaville et al., 2012a, 2012b; Tai et al., 2003), 30 min before surgery, animals were injected intraperitoneally with desipramine (25 mg/kg; Sigma) dissolved in 0.9% sodium chloride and injected at a volume of 5 ml/kg body weight. Rats were placed in a stereotaxic frame (Unimecanique, Paris, France) under xylazine/ketamine anesthesia (ketamine hydrochloride (75 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.), Sigma). Each animal received a unilateral injection of 2.5 μl of 6-OHDA (5 mg/ml in sterile NaCl, 0.9%; Sigma) with 0.01% ascorbic acid into the right medial forebrain bundle at coordinates 2.8 mm posterior to bregma, 2 mm lateral to the midline, and 8.4 mm below the skull according to the brain atlas of Paxinos and Watson (1996). The 6-OHDA injection was made over a 5 min period of time and then, the syringe needle was withdrawn slowly to prevent reflux of the solution. Sham rats received 2.5 μl of NaCl, 0.9% in the same conditions. Behavioral and electrophysiological studies have been done after a minimum recovery time of 3 weeks after 6-OHDA or saline injection.

Open-field locomotor activity

Spontaneous horizontal locomotion, vertical activity (or rearing) and stereotypic movements were measured using a photoelectric actimeter (Actitrack; Panlab, Barcelona, Spain), as previously described (Delaville et al., 2012a; Dulawa et al., 1999). The apparatus consisted of a transparent cage that was connected to a photoelectric cell. Light beams detected movement, and the total number of horizontal movements of each rat was recorded over two successive sessions of 10 min each day. All testing in the actimeter was done in an isolated room between 8:00 A.M. and 1:00 P.M. and consisted of three phases for each group of rats. In phase A, spontaneous locomotor activity was recorded during three consecutive days, immediately after local injection of NaCl 0.9% each day. Between-session habituation was analyzed by comparing behavior in the actimeter on day 2 versus that on day 3. The first session of 10 min was considered as the daily habituation. Only the locomotor activity recorded during the second session of 10 min was used for data analysis. In phase B, for drug injection, as for saline injection, the needle used for microinjection was 1 mm longer than the guide cannula and was connected to a 10-μl Hamilton digital syringe (Hamilton Company, Nevada, USA) through tubing. The needle was carefully inserted into the guide cannula and drugs were injected slowly over 2 min. 200 nl was selected after a series of control tests in which this volume showed a diffusion of the solution into the whole STN without a spread outside the nucleus. The needle was removed after a 60 s period. The rat was then immediately placed into the actimeter, and locomotor activity was recorded during two sessions of 10 min. In phase C, 1 day after noradrenergic agent injection, a post-challenge test was done and rats were re-exposed to the actimeter immediately after STN injection of 0.9% NaCl (day 5). If the values of the actimeter returned to the basal level, animals received local injection of another noradrenergic agent the day after. The experiments were performed in a total of 44 rats distributed in four groups and rats of each group received the injection of a maximum of two noradrenergic agents. Sham rats (n = 8) and 6-OHDA rats (n = 10) received the agonist and antagonist of α1-AR and the other groups of sham rats (n = 16) and 6-OHDA rats (n = 10) received the agonist and antagonist of α2-AR.

Extracellular recordings

Extracellular single-unit recordings were made in rats anesthetized with urethane (1.2 g/kg, i.p.). Recordings were done in sham and 6-OHDA-lesioned rats 3 weeks after surgery as previously reported (Delaville et al., 2012a, 2012b; Tai et al., 2003). Double or triple glass micropipettes, with one for recording (impedance, 8–12 MΩ) and one or two for drug injection were used. The tips between recording and injection micropipettes were separated by 160 to 220 nm. The injection pipette was filled with a NAergic drug and the recording pipette was filled with 4% Pontamine sky blue in 0.09% NaCl. The micropipettes were placed into the STN according to the coordinates given in the brain atlas of Paxinos and Watson (1996) (anteroposterior, 3.8 mm posterior to bregma; lateral, 2.5 mm from the midline; dorsal, 6.8–8.2 mm from the dura). Extracellular neuronal activity was amplified, bandpass-filtered (300–3000 Hz) using the Neurolog system (Digitimer, Hertfordshire, UK), displayed on an oscilloscope and transferred via a Powerlab interface to a computer equipped with Chart 5 software (AD Instruments, Charlotte, USA). Only neuronal activity with a signal-to-noise ratio of 3:1 was recorded and used for additional investigation. Basal firing of STN neurons was recorded for 20 min before drug

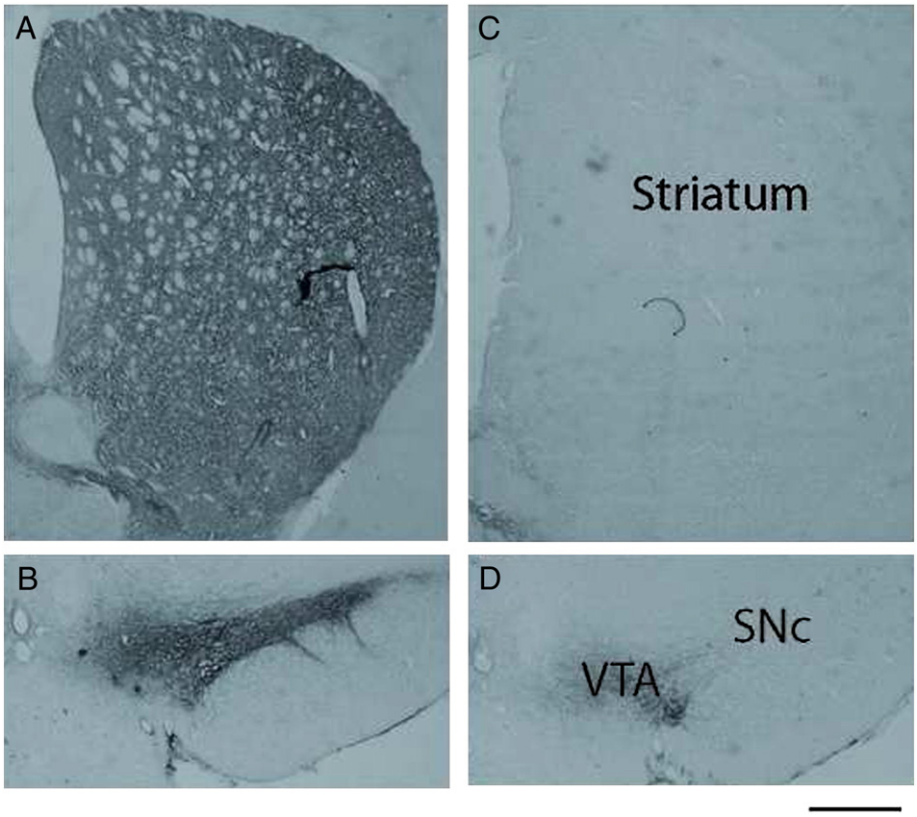

Fig. 1. Immunohistochemistry of tyrosine hydroxylase (TH) showing an almost total lesion of dopamine cell bodies in the substantia nigra pars compacta (SNc, D) and fibers in the striatum (C) after the injection of 6-OHDA into the MFB compared to sham-lesioned animals (A: striatum, B: SNc). Scale bar is 1 mm.

injection to ascertain the stability of the discharge activity, then a noradrenergic agent or the saline vehicle was injected directly into the STN at a volume of 20 nl, using brief pulses (200 ms) of pneumatic pressure (Picospritzer III, Royston Herts, UK). This small volume was used to avoid the risk of losing the recorded cell due to pressure effects. In 6-OHDA rats, STN neuronal activity was recorded in the 6-OHDA-lesioned hemisphere. In all tested rats, the central part of the nucleus was targeted.

At the end of each session, the recording site was marked by electrophoretic injection (Iso DAM 80; WPI, Hertfordshire, UK) of Pontamine sky blue through the micropipette at a negative current of 20 μA for 8 min. STN neuron activity was analyzed with a spike discriminator using a spike histogram program (AD Instruments, Charlotte, USA), and firing parameters were determined using the Neuroexplorer program (AlphaOmega, Nazareth, Israel).

Validation of the recording and implantation sites

After completion of the experiments, animals were sacrificed by an overdose of urethane, the brains removed, frozen in isopentane at −45 °C and stored at −80 °C. Fresh-frozen brains were cryostat-cut into 20 μm coronal sections and acetylcholine esterase staining was used as previously described (Chetrit et al., 2009) to visualize the guide cannula track and to determine the location of the Pontamine sky blue dots marking the recording site in the recorded structure. Only brains in which both the recording and drug injection were shown to be in the STN were used for data analysis.

Validation of DA lesion

Dopamine cell degeneration was determined by immunohistochemistry of tyrosine hydroxylase as previously described (Bouali-Benazzouz et al., 2009). Sections of the SNc and striatum were stained and only rats with almost total loss of TH immunoreactivity were used to analyze electrophysiological recordings and behavioral experiments (Fig. 1).

Statistical analysis

Statistical analyses were performed using Sigmaplot (Systat Software, San Jose, USA). For behavioral data, values were compared using a Friedman test followed, when significant, by Dunn's multiple post-tests. For electrophysiological data, normalized firing rates, before and after drug injection, were compared using a Friedman test followed, when significant, by Dunn's multiple post-tests. Firing patterns were analyzed using the method developed by Kaneoke and Vitek (1996) as previously described (Boraud et al., 1998; Tai et al., 2003) to determine if STN neurons discharged with regular, irregular or bursty pattern and the proportions of neurons according to their pattern were compared using a Chi^2 test. In addition, the coefficient of variation of the interspike intervals was analyzed to determine the changes in the firing pattern during time after drug injections using a Friedman test followed, when significant, by Dunn's multiple post-tests. The effects of clonidine alone or blocked by the previous injection of idazoxan were compared by a two way ANOVA followed, when significant, by a multiple comparison procedures using Student–Newman–Keuls test.

Results

Effects of STN α1- and α2-AR modulation on locomotor activity of sham and 6-OHDA rats

Spontaneous locomotor activity was measured using an "open field" actimeter as previously reported (Belujon et al., 2007; Delaville et al., 2012a). Preliminary studies showed that after 3 days of habituation, the behavior of the animals was stable (Student *t*-

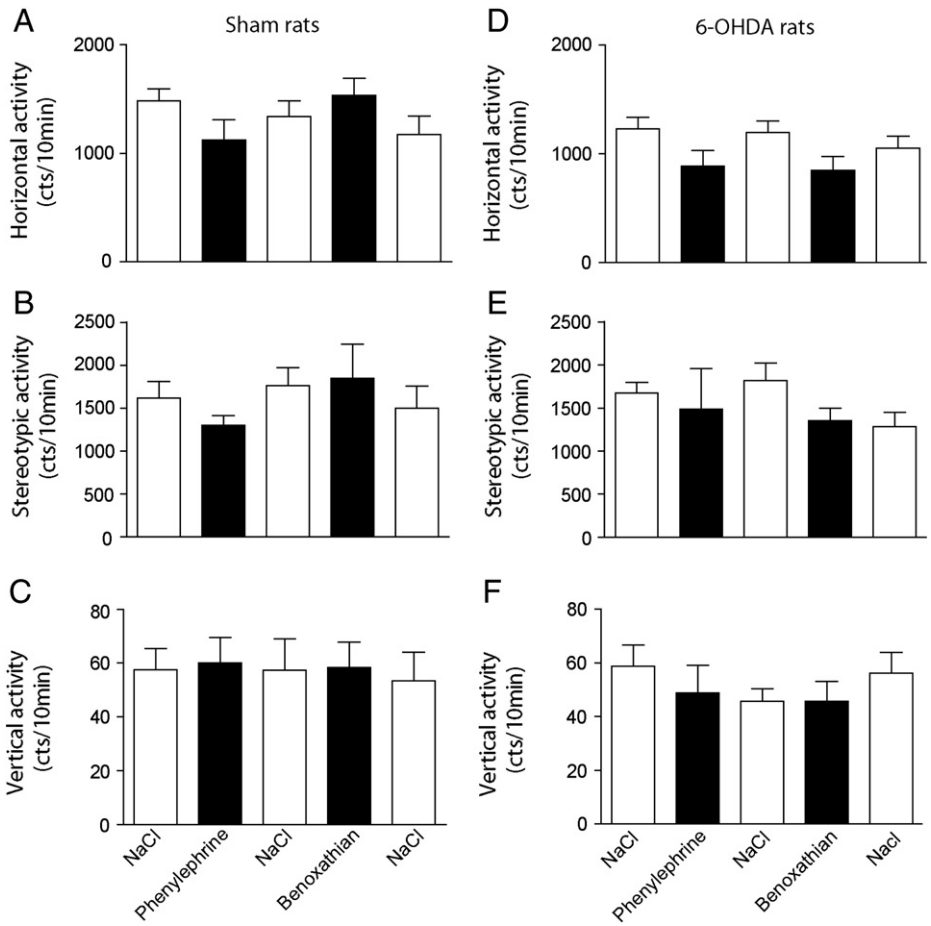

Fig. 2. Effects of α1-AR agonist and antagonist injected into the STN on locomotor activity of sham and 6-OHDA rats. Histograms represent the number of horizontal (A,D), stereotypic (B,E) and vertical (C,F) movements recorded during 10 min. Neither phenylephrine (α1-AR agonist) nor benoxathian (α1-AR antagonist) changed the number of the three movement parameters in both sham (n = 8, A–C) and 6-OHDA (n = 10, D–F) rats. Values are the mean ± SEM. Statistical analysis using Friedman test followed, when significant, by Dunn's multiple post tests was performed.

test, $p > 0.05$, ns), indicating that the rats were accustomed to their test environment (data not shown).

The effects of local injection of α1-AR agents into the STN on locomotor activity of sham rats and 6-OHDA rats are shown in Fig. 2. Administration of phenylephrine (α1-AR agonist) and benoxathian (α1-AR antagonist) did not modify horizontal locomotor activity in sham [$p > 0.05$, ns, Dunn's test after a significant Friedman test, $F = -42.92$, $p < 0.001$, $n = 8$, Fig. 2A] or in 6-OHDA rats [$p > 0.05$, ns, Dunn's test after a significant Friedman test, $F = -71.92$, $p < 0.001$, $n = 10$, Fig. 2D]. They have not changed neither stereotypic movements (sham: Friedman test, $F = 1.447$, $p > 0.05$, ns, $n = 8$, Fig. 2B; 6-OHDA: Friedman test, $F = 1.007$, $p > 0.05$, ns, $n = 10$, Fig. 2E) nor rearing (sham: Friedman test, $F = 0.141$, $p > 0.05$, ns, $n = 8$, Fig. 2C; 6-OHDA: Friedman test, $F = 1.285$, $p > 0.05$, ns, $n = 10$, Fig. 2F).

However, injection of clonidine (α2-AR agonist) into the STN significantly decreased horizontal locomotor activity compared with saline injection into the STN of sham [$p < 0.01$, Dunn's test after a significant Friedman test, $F = -19.24$, $p < 0.001$, $n = 16$, Fig. 3A] and 6-OHDA rats [$p < 0.01$, Dunn's test after a significant Friedman test, $F = -18.91$, $p < 0.001$, $n = 10$, Fig. 3D]. Similarly, Clonidine significantly decreased stereotypic movements [sham: $p < 0.001$, Dunn's test after a significant Friedman test, $F = 6.25$, $p < 0.01$, $n = 16$, Fig. 3B; 6-OHDA: *$p < 0.05$*, Dunn's test after a significant Friedman test, $F = 3.861$, $p < 0.05$, $n = 10$, Fig. 3E] and rearing [sham: $p < 0.01$, Dunn's test after a significant Friedman test, $F = 3.94$, $p < 0.05$, $n = 16$, Fig. 3C; 6-OHDA: *$p < 0.05$*, Dunn's test after a significant Friedman test, $F = 3.035$, $p < 0.05$, $n = 10$, Fig. 3E].

Idazoxan (α2-AR antagonist) did not induce any effect on horizontal locomotor activity in sham [$p > 0.05$, Dunn's test after a significant Friedman test, $F = -19.24$, $p < 0.001$, $n = 16$, Fig. 1C] or in 6-OHDA rats [$p > 0.05$, Dunn's test after a significant Friedman test, $F = -18.91$, $p < 0.001$, $n = 10$, Fig. 1D]. Similarly, Idazoxan did not affect neither stereotypic movements [sham: $p > 0.05$, Dunn's test after a significant Friedman test, $F = 6.25$, $p < 0.01$, $n = 16$, Fig. 3B; 6-OHDA: $p > 0.05$, Dunn's test after a significant Friedman test, $F = 3.861$, $p < 0.05$, $n = 10$, Fig. 3E] nor rearing [sham: $p > 0.05$, Dunn's test after a significant Friedman test, $F = 3.94$, $p < 0.05$, $n = 16$, Fig. 3C; 6-OHDA: *$p > 0.05$*, Dunn's test after a significant Friedman test, $F = 3.035$, $p < 0.05$, $n = 10$, Fig. 3E].

Effects of STN α1- and α2-AR modulation on the firing activity of STN neurons in sham and 6-OHDA rats

Table 1 summarizes the firing rates and coefficient of variations of the interspike interval of STN neurons before and 10 min after drug injections. Injection of the α1-AR agonist, phenylephrine, into the STN induced a dramatic increase in the firing rate of STN neurons in sham [$p < 0.001$, Dunn's test after a significant Friedman test, $F = 67.73$, $p < 0.001$, $n = 27$, Figs. 4A,B] as well as in 6-OHDA rats [$p < 0.001$, Dunn's test after a significant Friedman test, $F = 37.03$; $p < 0.001$, $n = 11$, Figs. 4E,F]. This effect occurred 10 min after the injection and lasted 30 min. In contrast, no change was observed after the α1-AR antagonist, benoxathian was injected in sham [Friedman test, $F = 7.25$, $p > 0.05$, ns, $n = 6$, Figs. 4A,B] or in 6-OHDA rats

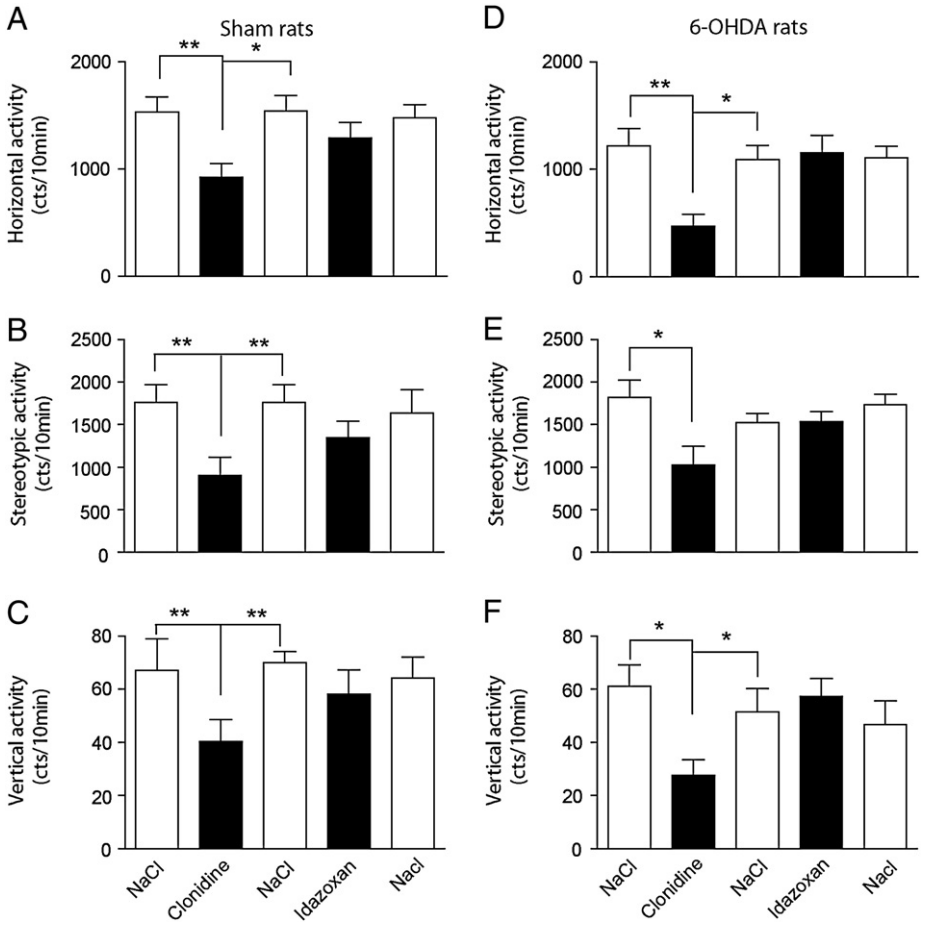

Fig. 3. Effects of α2-AR agonist and antagonist injected into the STN on locomotor activity of sham and 6-OHDA rats. Histograms represent the number of horizontal (A,D), stereotypic (B,E) and vertical (C,F) movements recorded during 10 min. Clonidine (α2-AR agonist) significantly decreased the number of the three movement parameters in both sham (n = 16) and 6-OHDA (n = 10) rats. However, idazoxan (α2-AR antagonist) did not change these parameters in the two groups of rats. Values are the mean ± SEM. Statistical analysis using Friedman test followed, when significant, by Dunn's multiple post tests was performed. * $p<0.05$, ** $p<0.01$.

[$p>0.05$, Dunn's test after a significant Friedman test, F = 11.24, $p<0.05$, n = 9, Figs. 4E,F].

As regards the firing pattern, neither phenylephrine nor benoxathian induced a change in sham or 6-OHDA rats (Figs. 4C,D and Figs. 4G,H respectively). Indeed, the coefficient of variation of the interspike interval of STN neurons was not modified after phenylephrine injection into the STN of sham [Friedman test, F = 16.54, $p>0.05$, ns, n = 27, Fig. 4C] or 6-OHDA rats [Friedman test, F = 8.824, $p>0.05$, n = 11, Fig. 4G]. Similarly, benoxathian did not modify the coefficient of variation in either groups [sham rats: Friedman test, F = 0.76, $p>0.05$, ns, n = 6, Fig. 4C; 6-OHDA rats: Friedman test, F = 2.376, $p>0.05$, ns, n = 9, Fig. 4G].

Table 1
Changes in firing rates (A) and coefficient of variations of the interspike intervals (ISI) (B) induced by the local injection of noradrenergic agents into the STN of sham and 6-OHDA-lesioned rats. The values are mean ± SEM. obtained before and 10 min after drug injections.

	Sham		6-OHDA	
	Before	After	Before	After
A. Firing rates (spikes/s)				
Phenylephrine	13.6 ± 1.73	24 ± 3.5***	12.6 ± 2.1	16.8 ± 2.56***
Benoxathian	10.4 ± 1.5	11.9 ± 2.7	11.1 ± 2.95	12.5 ± 3.9
Clonidine	12.3 ± 2.04	10.87 ± 1.75	9.9 ± 1.02	9.52 ± 1.29
Idazoxan	10.95 ± 2.97	9.98 ± 2.82	10.79 ± 2.07	11.54 ± 1.36
B. Coefficient of variations of the ISI				
Phenylephrine	0.59 ± 0.08	0.56 ± 0.06	1.28 ± 0.24	1.25 ± 0.24
Benoxathian	0.68 ± 0.17	0.68 ± 0.2	0.83 ± 0.19	0.68 ± 0.16
Clonidine	0.65 ± 0.09	1.55 ± 0.5**	0.92 ± 0.15	1.38 ± 0.25***
Idazoxan	0.67 ± 0.11	0.75 ± 0.13	0.93 ± 0.28	0.93 ± 0.27

Statistical analysis using a Friedman test followed, when significant, by Dunn's multiple comparison test was performed.
*** $p<0.001$, in comparison with basal activity.
** $p<0.01$, in comparison with basal activity.

Regarding the α2-AR, STN injection of clonidine did not induce a significant change in the firing rate of STN neurons in sham [Friedman test, F = 3.07, $p>0.05$, ns, n = 16, Figs. 5A,B] or in 6-OHDA rats [Friedman test, F = 3.84, $p>0.05$, ns, n = 11, Figs. 5E,F]. As was found for the α1-AR, no change in the firing rate was observed after the α2-AR antagonist injection, idazoxan, in sham [Friedman test, F = 2.24, $p>0.05$, ns, n = 7, Figs. 5A,B] or in 6-OHDA rats [Friedman test, F = 1.86, $p>0.05$, ns, n = 7, Figs. 5E,F].

Although idazoxan did not induce any change in the firing pattern of STN neurons in sham [Friedman test, F = 0.57, $p>0.05$, ns, n = 7, Figs. 5C,D] or in 6-OHDA rats [Friedman test, F = 1.83, $p>0.05$, ns, n = 7, Figs. 5G,H], clonidine significantly increased the percentage of neurons discharging with burst in sham (Chi^2 test, $p<0.01$, Fig. 5D) as well as in 6-OHDA rats (Chi^2 test, $p<0.001$, Fig. 5H). Indeed, the coefficient of variation of interspike interval of STN neurons significantly increased after clonidine injection into the STN of sham [$p<0.01$, Dunn's test after a significant Friedman test, F = 37.02; $p<0.001$, n = 16, Fig. 5C] and 6-OHDA rats [$p<0.001$, Dunn's test after a significant Friedman test, F = 36.12; $p<0.001$, n = 11, Fig. 5G]. This effect occurred 10 min after the injection and no recovery to the basal level was observed before 40 min after the injection. The selectivity of clonidine on α2-ARs was tested by injecting idazoxan 5 min before its injection. In this situation, the increased coefficient of variation

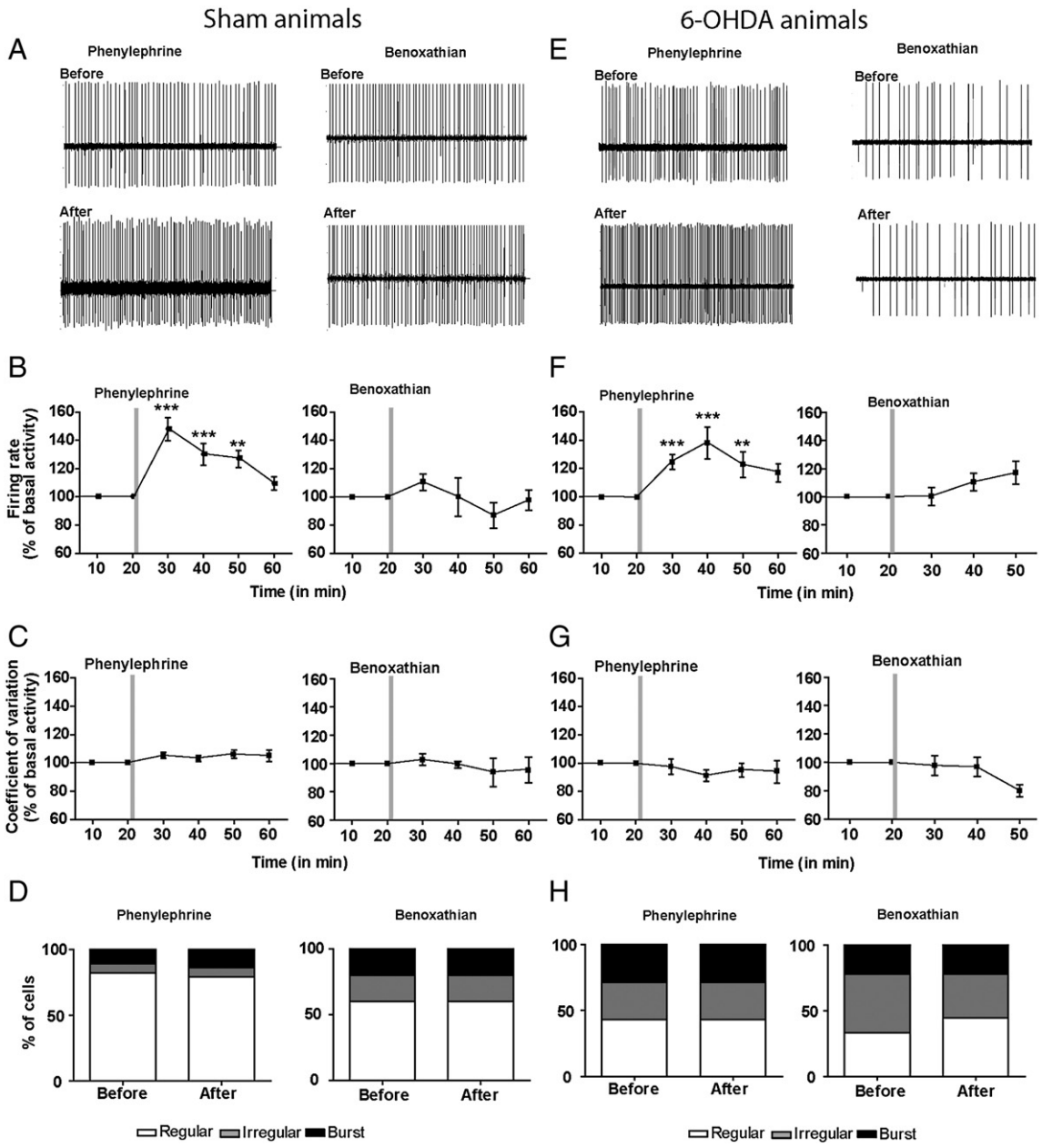

Fig. 4. Effects of α1-AR agonist and antagonist injected into the STN on the firing activity of STN neurons in sham and 6-OHDA rats. A–D: effects of α1-AR agents in sham rats. E–H: effects of α1-AR agents in 6-OHDA rats. A and E: section of recordings with representative examples of STN neuronal activity before and after the injection of α1-AR drugs. B and F: phenylephrine (α1-AR agonist) significantly increased the firing rate of STN neurons in sham (n = 27 cells) and 6-OHDA (n = 11 cells) rats, however, benoxathian (α2-AR antagonist) did not change the firing rate of STN neurons in either group (n = 6 cells in sham rats; n = 9 in 6-OHDA rats). Values are the percentage of basal activity ± SEM. C and G: phenylephrine, like benoxathian, did not induce any significant change in the coefficient of variation. Values are the percentage of basal activity ± SEM. D and H: firing pattern histograms showing the absence of changes in the proportion of STN neurons discharging regularly, irregularly or with bursts after the injection of α1-AR drugs in the two groups. Statistical analysis using Friedman test followed, when significant, by Dunn's multiple comparison test was performed for the firing rate and the coefficient of variation. Chi^2 test was performed for the firing patterns. ** $p<0.01$, *** $p<0.001$. Time scale for A and E: 5 s duration of each trace.

induced by clonidine alone was prevented by the prior injection of idazoxan [$p<0.01$, Newman–Keuls test after a two way ANOVA, $F_{(125,5)} = 1.45$, $p<0.02$, Fig. 6].

Saline injection into the STN did not change either the firing rate or the firing pattern of STN neurons (Friedman test, $F = 0.466$, ns, $n = 5$ for the firing rate; $F = 1.905$, $p>0.05$, ns, $n = 5$ for the coefficient of variation of intespike intervals).

Discussion

The present study provides the first in vivo evidence that activation of α2-ARs, located in the STN increased the ability of STN neurons to discharge in bursts without affecting the firing rate and subsequently decreased locomotor activity in both sham and 6-OHDA rats. However, activation of STN α1-ARs, which increased the firing rate of STN neurons without affecting the firing pattern, did not affect locomotor activity in the two groups of animals. This is the first direct evidence supporting the possibility that noradrenaline acting on α2-AR contributes to the emergence of burst firing pattern of STN neurons, which is a pathological signature related to parkinsonian motor disabilities.

Implication of the STN in motor control

Many studies have identified the STN as a basal ganglia nucleus playing a key role in motor control and consequently in the

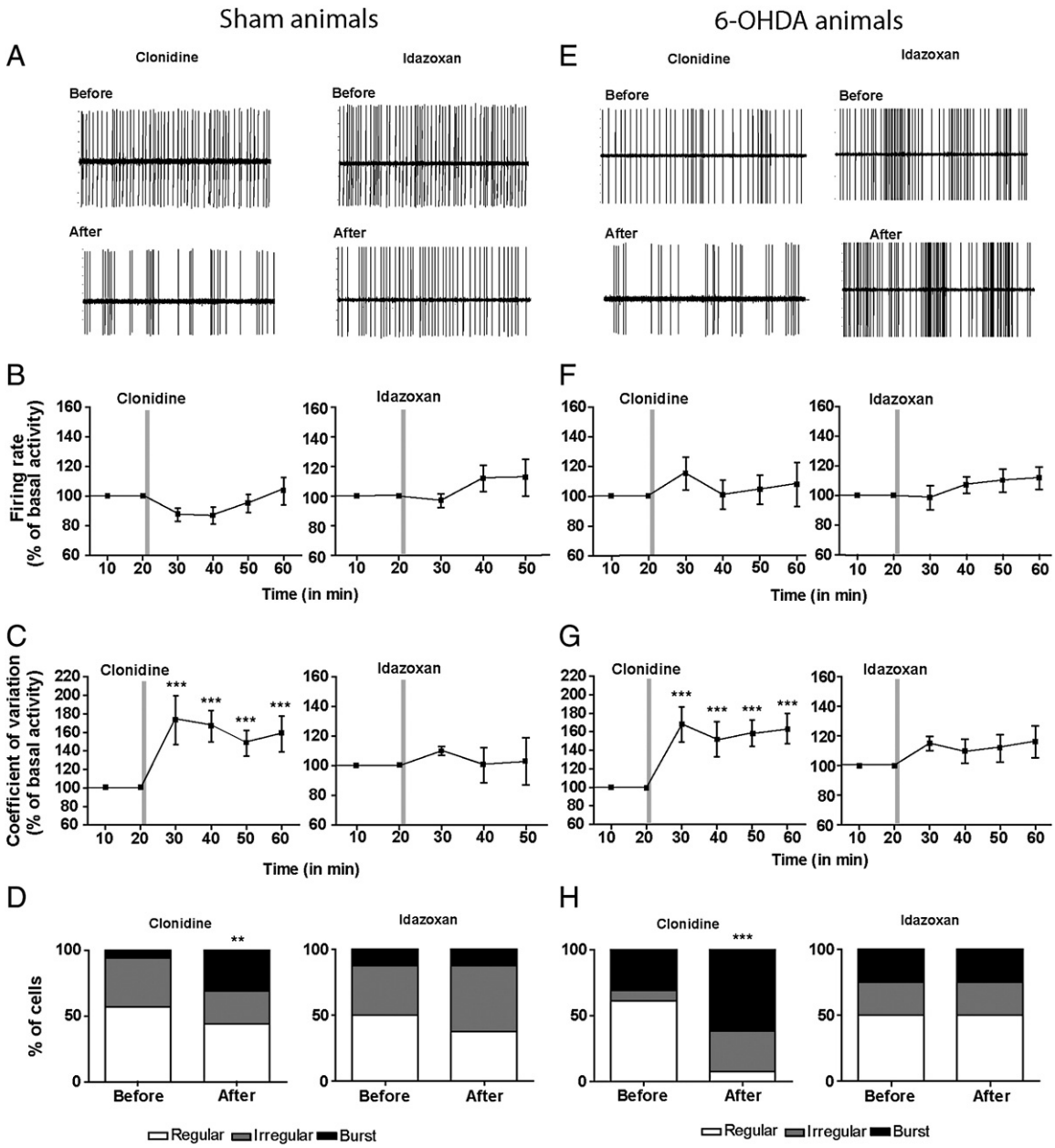

Fig. 5. Effects of α2-AR agonist and antagonist injected into the STN on the firing activity of STN neurons in sham and 6-OHDA rats. A–D: effects of α2-AR agents in sham rats. E–H: effects of α2-AR agents in 6-OHDA rats. A and E: section of recordings with representative examples of STN neuronal activity before and after the injection of α2-AR drugs. B and F: neither clonidine (α2-AR agonist) nor idazoxan (α2-AR antagonist) changed the firing rate of STN neurons in sham (n = 16 and n = 7 cells respectively) and 6-OHDA (n = 11 and n = 7 cells respectively) rats. Values are the percentage of basal activity ± SEM. C and G: clonidine induced a significant increase in the coefficient of variation of interspike intervals, however, idazoxan did not induce any change in this coefficient. Values are the percentage of basal activity ± SEM. D and H: firing pattern histograms showing that clonidine induced a significant increase in the proportion of bursty neurons in both sham and 6-OHDA rats, however, idazoxan did not change these proportions. Statistical analysis using Friedman test followed, when significant, by Dunn's multiple comparison test was performed for the firing rate and the coefficient of variation. Chi^2 test was performed for the firing patterns. ** $p<0.01$, *** $p<0.001$. Time scale for A and E: 5 s duration of each trace.

pathophysiology of PD. Indeed, after DA depletion, STN neurons, which normally exhibit a tonic discharge pattern, become bursty in animal models of PD (Bergman et al., 1994; Ni et al., 2001). This pathological bursty pattern has also been reported in PD patients (Benazzouz et al., 2002; Delaville et al., 2011; Hutchison et al., 1998). Moreover, the motor symptoms of PD are improved by either STN ablation (Bergman et al., 1990; Guridi and Obeso, 2001) or high frequency stimulation (Benabid et al., 2000; Benazzouz et al., 1993). The STN is a small but complex structure receiving afferent projections from different brain areas. STN neuronal activity is under the control of GABAergic afferents from the globus pallidus (Parent and Hazrati, 1995), glutamatergic afferents from the cortex (Kitai and Deniau, 1981; Parent and Hazrati, 1995), dopaminergic afferents from the SNc (Baufreton et al., 2003; Cragg et al., 2004; Hassani and Feger, 1999; Ni et al., 2001) and serotonergic afferents from the dorsal raphe nucleus (Stanford et al., 2005; Xiang et al., 2005). In addition to these afferents, the STN receives also noradrenergic fibers arising from locus coeruleus (Canteras et al., 1990; Carpenter et al., 1981; Parent and Hazrati, 1995). However, NA modulation of STN neuronal activity is not documented (Arcos et al., 2003; Belujon et al., 2007).

STN α2-ARs but not α1-ARs are involved in the control of locomotor activity

Our results show that α1-ARs located in the STN are not implicated in the control of locomotor activity. Indeed, neither activation nor

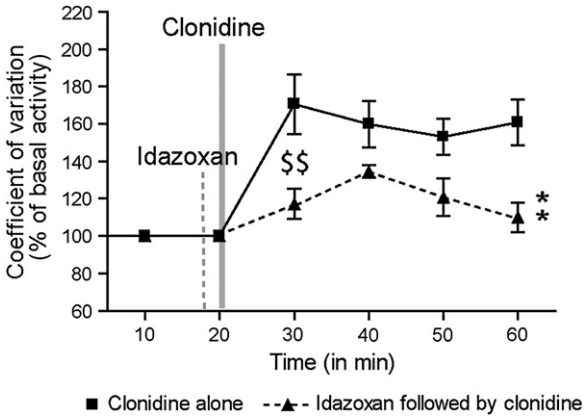

Fig. 6. Verification of the selective effect of clonidine on α2-ARs Idazoxan when injected before clonidine, it prevented the changes in the coefficient of variation of interspike intervals induced by clonidine alone. Statistical analysis using a two way ANOVA was performed, ** $p<0.02$ for idazoxan followed by clonidine versus clonidine alone. Newman–Keuls test, $^{\$\$}$ $p<0.01$ at time point 30 min.

blockade of these receptors had a clear effect upon the locomotor activity parameters in both sham and lesioned rats. Interestingly, we show that α2-ARs are implicated in the motor control as their activation by the agonist clonidine decreased the spontaneous locomotor activity of both sham and lesioned rats, i.e. displaying a parkinsonian-like hypokinesia. This hypo-locomotor activity is not due to a cataleptic state as the parameters of the bar test were not modified after the injection of clonidine into the STN. However, blockade of α2-ARs by the antagonist idazoxan did not show any change in the spontaneous locomotor activity parameters. These results suggest that the changes in locomotor activity induced by systemic injection of α1- and α2-AR agonists/antagonists reported in previous studies (Antonelli et al., 1991; Belujon et al., 2007; Mathe et al., 1996; Wellman and Davies, 1992) are not due to their direct effect on STN neurons, but to an indirect network effect.

Activation of α1-ARs located in the STN induced changes in STN firing rate whereas activation of α2-ARs induced changes in STN firing pattern

Effects of STN α1-AR modulation

We show that α1-ARs located in the STN are implicated in the modulation of the STN neuronal firing rate. Indeed, their activation by the agonist increased the firing rate without changing the firing pattern of STN neurons in both sham and lesioned rats. Moreover, the firing rate increase was not paralleled with a change in locomotor activity. These results provide direct evidence that the increase in the firing rate of STN neurons is not related to the induction of motor hypokinesia. These "excitatory" α1-ARs, which are coupled to a Gq protein (McCormick and Wang, 1991; McCormick et al., 1991), are located on the postsynaptic membrane in different brain nuclei (Lakhlani et al., 1996; Langer, 1974; Starke, 1972) including the STN (Belujon et al., 2007). Our electrophysiological results fit with previous in vitro studies showing the same excitatory effect in STN slices (Arcos et al., 2003) even after blocking afferent synaptic transmission (Arcos et al., 2003). Moreover, the observed effect is not mediated by DA transmission as we show that DA depletion did not influence the responses of STN neurons induced by α1-AR drugs.

Effects of STN α2-AR modulation

In contrast to α1-ARs, activation of α2-ARs, which decreased locomotor activity, dramatically increased the capacity of STN neurons to discharge in bursts without changing the firing rate. This represents direct evidence that the bursty pattern, not the increase in the firing rate, of STN neurons is the most relevant pathological activity related to the manifestation of hypokinesia. These results are in agreement with our previous in vitro study in which an α2-AR agonist was tested on evoked plateau potentials of STN neurons. We have shown that α2-AR activation increased all indices of burst competency (Belujon et al., 2007). The bursty pattern induced by the activation of α2-ARs is due to a presynaptic effect as these "inhibitory" receptors, coupled to Gi proteins, are located on afferent nerve terminals in different brain nuclei (Benarroch, 2009; Lakhlani et al., 1996; Langer, 1974; Starke, 1972) including the STN (Belujon et al., 2007). The presynaptic effect of the α2-AR agonist is in agreement with the results of Arcos et al. (2003), who studied the effects of α2-AR activation in vitro in STN slices. After blockade of synaptic transmission, these authors showed that α2-AR activation did not induce any change in STN neuronal activity. Furthermore, it is unlikely that the effect induced by clonidine may be due to its action on receptors other than α2-ARs, as the α2-AR antagonist treatment, idazoxan prevented the firing pattern changes induced by clonidine. In contrast to the results obtained in vitro showing that idazoxan reduced all indices of burst competency (Belujon et al., 2007), we show that, even if this α2-AR antagonist prevented the effect of the agonist, it did not change the firing activity of STN neurons when injected alone. This discrepancy could be explained by the fact that in vitro idazoxan was tested on evoked plateau potentials, not on the spontaneous activity of STN neurons as in the present study.

Functional relevance of STN α2-ARs

Although it is known that α2-ARs are located presynaptically on afferents to STN, it is not clear on which class of neurons they are located. The three main classes of afferents arise from cortex/thalamus, GPe and SNc. As we show that the activation of α2-ARs induced the same effect in sham and 6-OHDA rats, it is unlikely that these receptors are located on dopaminergic terminals arising from the SNc. These results fit with a previous study showing that atipamezole, an α2-AR antagonist, had no effect on dopamine overflow (Yavich et al., 2003). The absence of changes in the firing rate after clonidine injection might suggest a simultaneous activation of α2 ARs located on glutamatergic and GABAergic afferents, which could suppress transmitter release in both glutamatergic and GABAergic synapses. Although it has been demonstrated that α2-AR activation by clonidine inhibits the release of glutamate in several brain regions (Kamisaki et al., 1992, 1993), several studies have shown that clonidine was able to potentiate the effects of GABA, including in the red nucleus (Ciranna et al., 2000) and the vestibular nucleus (Di Mauro et al., 2008). Indeed, recent evidence suggests that emergent pathological bursty activity in the STN is probably due to an imbalance between glutamatergic and GABAergic transmission in the STN (Bevan et al., 2006).

In conclusion, this study adds noradrenaline to the list of neuroactive substances able to modulate the firing activity of STN neurons. Indeed, our data provide evidence that α2-ARs located in the STN play a key role in the modulation of STN firing pattern and that their activation is implicated in the genesis of STN bursty activity, which may be at the origin of parkinsonian motor deficits. Furthermore, our results reinforce the rationale for using α2-AR noradrenergic agents in the treatment of parkinsonian-like motor symptoms in addition to dopaminergic drugs.

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References

Antonelli, T., et al., 1991. Effect of alpha-adrenoreceptors in the control of spontaneous motility and morphine withdrawal syndrome. Boll. Soc. Ital. Biol. Sper. 67, 965–971.

Arcos, D., et al., 2003. Noradrenaline increases the firing rate of a subpopulation of rat subthalamic neurones through the activation of alpha 1-adrenoceptors. Neuropharmacology 45, 1070–1079.

Baufreton, J., et al., 2003. D5 (not D1) dopamine receptors potentiate burst-firing in neurons of the subthalamic nucleus by modulating an L-type calcium conductance. J. Neurosci. 23, 816–825.

Belujon, P., et al., 2007. Noradrenergic modulation of subthalamic nucleus activity: behavioral and electrophysiological evidence in intact and 6-hydroxydopamine-lesioned rats. J. Neurosci. 27, 9595–9606.

Benabid, A.L., et al., 2000. Deep brain stimulation of the subthalamic nucleus for Parkinson's disease: methodologic aspects and clinical criteria. Neurology 55, S40–S44.

Benarroch, E.E., 2009. The locus ceruleus norepinephrine system: functional organization and potential clinical significance. Neurology 73, 1699–1704.

Benazzouz, A., et al., 1993. Reversal of rigidity and improvement in motor performance by subthalamic high-frequency stimulation in MPTP-treated monkeys. Eur. J. Neurosci. 5, 382–389.

Benazzouz, A., et al., 2002. Intraoperative microrecordings of the subthalamic nucleus in Parkinson's disease. Mov. Disord. 17 (Suppl. 3), S145–S149.

Bergman, H., et al., 1990. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 249, 1436–1438.

Bergman, H., et al., 1994. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J. Neurophysiol. 72, 507–520.

Bertrand, E., et al., 1997. Qualitative and quantitative analysis of locus coeruleus neurons in Parkinson's disease. Folia Neuropathol. 35, 80–86.

Bevan, M.D., et al., 2006. Cellular principles underlying normal and pathological activity in the subthalamic nucleus. Curr. Opin. Neurobiol. 16, 621–628.

Bezard, E., et al., 1999. Effect of the alpha 2 adrenoreceptor antagonist, idazoxan, on motor disabilities in MPTP-treated monkey. Prog. Neuropsychopharmacol. Biol. Psychiatry 23, 1237–1246.

Boraud, T., et al., 1998. Effects of L-DOPA on neuronal activity of the globus pallidus externalis (GPe) and globus pallidus internalis (GPi) in the MPTP-treated monkey. Brain Res. 787, 157–160.

Bouali-Benazzouz, R., et al., 2009. Intrapallidal injection of 6-hydroxydopamine induced changes in dopamine innervation and neuronal activity of globus pallidus. Neuroscience 164, 588–596.

Boyajian, C.L., et al., 1987. Anatomical evidence for alpha-2 adrenoceptor heterogeneity: differential autoradiographic distributions of [3H]rauwolscine and [3H] idazoxan in rat brain. J. Pharmacol. Exp. Ther. 241, 1079–1091.

Canteras, N.S., et al., 1990. Afferent connections of the subthalamic nucleus: a combined retrograde and anterograde horseradish peroxidase study in the rat. Brain Res. 513, 43–59.

Carpenter, M.B., et al., 1981. Connections of the subthalamic nucleus in the monkey. Brain Res. 224, 1–29.

Chetrit, J., et al., 2009. Involvement of Basal Ganglia network in motor disabilities induced by typical antipsychotics. PLoS One 4, e6208.

Chopin, P., et al., 1999. Effects of alpha-2 adrenoceptor agonists and antagonists on circling behavior in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway. J. Pharmacol. Exp. Ther. 288, 798–804.

Ciranna, L., et al., 2000. Neurotransmitter-mediated control of neuronal firing in the red nucleus of the rat: reciprocal modulation between noradrenaline and GABA. Exp. Neurol. 163, 253–263.

Colpaert, F.C., 1987. Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. Neuropharmacology 26, 1431–1440.

Cragg, S.J., et al., 2004. Synaptic release of dopamine in the subthalamic nucleus. Eur. J. Neurosci. 20, 1788–1802.

Crestani, C.C., et al., 2008. Both alpha1 and alpha2-adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the bed nucleus of the stria terminal of rats. Br. J. Pharmacol. 153, 583–590.

Delaville, C., et al., 2011. Noradrenaline and Parkinson's disease. Front. Syst. Neurosci. 5, 31.

Delaville, C., et al., 2012a. Emerging dysfunctions consequent to combined monoaminergic depletions in parkinsonism. Neurobiol. Dis. 45, 763–773.

Delaville, C., et al., 2012b. Effects of noradrenaline and serotonin depletions on the neuronal activity of globus pallidus and substantia nigra pars reticulata in experimental parkinsonism. Neuroscience 202, 424–433.

Di Mauro, M., et al., 2008. Noradrenaline modulates neuronal responses to GABA in vestibular nuclei. Neuroscience 153, 1320–1331.

Dulawa, S.C., et al., 1999. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J. Neurosci. 19, 9550–9556.

Ehringer, H., Hornykiewicz, O., 1960. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Klin. Wochenschr. 38, 1236–1239.

Fornai, F., et al., 2007. Noradrenaline in Parkinson's disease: from disease progression to current therapeutics. Curr. Med. Chem. 14, 2330–2334.

Gomez-Mancilla, B., Bedard, P.J., 1993. Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. Clin. Neuropharmacol. 16, 418–427.

Guridi, J., Obeso, J.A., 2001. The subthalamic nucleus, hemiballismus and Parkinson's disease: reappraisal of a neurosurgical dogma. Brain 124, 5–19.

Hassani, O.K., Feger, J., 1999. Effects of intrasubthalamic injection of dopamine receptor agonists on subthalamic neurons in normal and 6-hydroxydopamine-lesioned rats: an electrophysiological and c-Fos study. Neuroscience 92, 533–543.

Hassani, O.K., et al., 1996. Increased subthalamic neuronal activity after nigral dopaminergic lesion independent of disinhibition via the globus pallidus. Neuroscience 72, 105–115.

Hutchison, W.D., et al., 1998. Neurophysiological identification of the subthalamic nucleus in surgery for Parkinson's disease. Ann. Neurol. 44, 622–628.

Kamisaki, Y., et al., 1992. Presynaptic inhibition by clonidine of neurotransmitter amino acid release in various brain regions. Eur. J. Pharmacol. 217, 57–63.

Kamisaki, Y., et al., 1993. Presynaptic alpha 2 adrenoceptors inhibit glutamate release from rat spinal cord synaptosomes. J. Neurochem. 60, 522–526.

Kaneoke, Y., Vitek, J.L., 1996. Burst and oscillation as disparate neuronal properties. J. Neurosci. Methods 68, 211–223.

Kitai, S.T., Deniau, J.M., 1981. Cortical inputs to the subthalamus: intracellular analysis. Brain Res. 214, 411–415.

Lakhlani, P.P., et al., 1996. Genetic evidence for involvement of multiple effector systems in alpha 2A-adrenergic receptor inhibition of stimulus-secretion coupling. Mol. Pharmacol. 50, 96–103.

Langer, S.Z., 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23, 1793–1800.

Mathe, J.M., et al., 1996. Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. Eur. J. Pharmacol. 309, 1–11.

Mavridis, M., et al., 1991. Differential modulation of (+)-amphetamine-induced rotation in unilateral substantia nigra-lesioned rats by alpha 1 as compared to alpha 2 agonists and antagonists. Brain Res. 562, 216–224.

McCormick, D.A., Wang, Z., 1991. Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. J. Physiol. 442, 235–255.

McCormick, D.A., et al., 1991. Actions of norepinephrine in the cerebral cortex and thalamus: implications for function of the central noradrenergic system. Prog. Brain Res. 88, 293–305.

Ni, Z.G., et al., 2001. Time-course of changes in firing rates and firing patterns of subthalamic nucleus neuronal activity after 6-OHDA-induced dopamine depletion in rats. Brain Res. 899, 142–147.

Parent, A., Hazrati, L.N., 1995. Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. Brain Res. Brain Res. Rev. 20, 128–154.

Parr-Brownlie, L.C., et al., 2007. Dopamine lesion-induced changes in subthalamic nucleus activity are not associated with alterations in firing rate or pattern in layer V neurons of the anterior cingulate cortex in anesthetized rats. Eur. J. Neurosci. 26, 1925–1939.

Paxinos, G., Watson, C., 1996. The Rat Brain in Stereotatic Coordinates. Academic Press, San Diego.

Stanford, I.M., et al., 2005. 5-Hydroxytryptamine induced excitation and inhibition in the subthalamic nucleus: action at 5-HT(2C), 5-HT(4) and 5-HT(1A) receptors. Neuropharmacology 49, 1228–1234.

Starke, K., 1972. Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: evidence for an -receptor-mediated feed-back inhibition of noradrenaline release. Naunyn Schmiedebergs Arch. Pharmacol. 275, 11–23.

Swanson, L.W., Hartman, B.K., 1975. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J. Comp. Neurol. 163, 467–505.

Tai, C.H., et al., 2003. Electrophysiological and metabolic evidence that high-frequency stimulation of the subthalamic nucleus bridles neuronal activity in the subthalamic nucleus and the substantia nigra reticulata. FASEB J. 17, 1820–1830.

Wang, R., et al., 1996. Expression of alpha 2-adrenergic receptor subtypes in the mouse brain: evaluation of spatial and temporal information imparted by 3 kb of 5′ regulatory sequence for the alpha 2A AR-receptor gene in transgenic animals. Neuroscience 74, 199–218.

Wellman, P.J., Davies, B.T., 1992. Effects of the alpha 1-adrenergic agonist cirazoline on locomotion and brown adipose tissue thermogenesis in the rat. Life Sci. 50, 1745–1753.

Xiang, Z., et al., 2005. Modulation of spontaneous firing in rat subthalamic neurons by 5-HT receptor subtypes. J. Neurophysiol. 93, 1145–1157.

Yavich, L., et al., 2003. Atipamezole, an alpha2-adrenoceptor antagonist, augments the effects of L-DOPA on evoked dopamine release in rat striatum. Eur. J. Pharmacol. 462, 83–89.