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Research report

Behavioral sensitization to different dopamine agonists in a parkinsonian rodent model of drug-induced dyskinesias

M.A. Delfino [a,*], A.V. Stefano [a], J.E. Ferrario [a], I.R.E. Taravini [a], M.G. Murer [b], O.S. Gershanik [a]

[a] *Laboratorio de Parkinson Experimental, ININFA-CONICET, Junín 956, 5° Piso, C1113AAD Buenos Aires, Argentina*
[b] *Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*

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Abstract

Repeated treatment with dopamine (DA) receptor agonists strongly potentiates contralateral turning behavior due to selective stimulation of D1 or D2-class receptors in 6-hydroxydopamine (6-OHDA)-lesioned rats. This phenomenon, referred to as sensitization, is believed to be related to the motor response complications (dyskinesias, on-off states) that occur during chronic administration of levodopa in Parkinson's disease patients. In recent years a new method for the evaluation of abnormal involuntary movements (AIMs) secondary to dopaminergic stimulation in 6-OHDA-lesioned rats was described. These AIMs resemble dyskinesias as seen in parkinsonian patients under levodopa therapy. Our objective was to evaluate the effects of repeated treatment with different regimes of DA agonists on turning behavior and on an AIMs scale in 6-OHDA lesioned rats, with the aim of discriminating between drugs with different dyskinesia-inducing potential. In addition, we explored the effects of a previous exposure to a DA agonist (priming) on the behavioral response to the subsequent administration of a DA agonist with the same or different pharmacologic profile. Our results show that in apomorphine-treated rats, rotational behavior and AIMs run a parallel course of enhancement, while in those receiving quinpirole there is a dissociation, suggesting that they could be mediated by different mechanisms. The finding of a significant priming effect on subsequent testing of 6-OHDA lesioned rats should be borne in mind as the use of these pharmacological tests in the screening of well lesioned animals could lead to an erroneous interpretation of further results on dyskinesias and rotational behavior.

Keywords: Parkinson's disease; Priming; Abnormal involuntary movements; 6-OHDA-lesioned rats; Rotational behavior; Sensitization

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by bradykinesia, tremor, rigidity, and postural abnormalities. PD patients treated chronically with levodopa (L-Dopa), frequently develop involuntary movements (dyskinesias) and response fluctuations, being at present one of the major limitations of PD therapy. The basis of these motor complications is unknown, and the strategies to prevent them are a subject of discussion. Dyskinesias have been associated with a sequence of events that include pulsatile stimulation of striatal dopamine (DA) receptors, downstream changes in gene expression, and abnormalities in non-DA transmitter systems [4]. Animal studies show that L-Dopa-induced dyskinesia (LID) and DA-dependent stereotypies are associated with an induction of c-*fos* family genes in striatal projection neurons [11].

Rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion in the dopaminergic nigrostriatal pathway show behavioral sensitization upon repeated treatment with DA agonists [27]. Numerous studies show that a single exposure (priming) to a DA receptor agonist greatly enhances the contralateral turning behavior elicited by subsequent challenges with DA agonists [10]. In PD patients, the priming effect is also observed once they have been exposed to L-Dopa, and is one of the factors leading towards the development of motor response complications whether they are treated afterwards with L-Dopa or a D2 agonist [32].

Enhancement of the rotational response is used routinely as an index of the ability of dopaminergic drugs to induce motor complications [14,15,28,34]. In recent publications, manipulation of the rotational response has been proposed

* Corresponding author. Tel.: +54-11-4961-5949/6784; fax: +54-11-4963-8593.
E-mail address: madelfi@ffyb.uba.ar (M.A. Delfino).

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as a tool to explore different treatment modalities capable of reducing dyskinesia [14].

Recent findings show that 6-OHDA-lesioned rats do in fact exhibit motor deficits and abnormal involuntary movements (AIMs) of the axial musculature and contralateral forelimb sharing functional similarities with parkinsonian akinesia or dyskinesia [9,16,17,35]. Lundblad et al. [17] found that while contralateral rotational behavior was increased during a course of drug treatment, the rat's ability to perform purposeful motor acts either remained stable or declined depending if the drug treatment was with bromocriptine or with L-Dopa, respectively.

There is therefore an ongoing discussion as to which behavioral test in rats can better measure both the therapeutic effect and the dyskinesia-inducing potential of a given DA agonist. Not all dopaminergic agents share the same capacity to induce dyskinesias in clinical practice, and the ability to discriminate among them using a simple screening animal model would be of major practical importance.

Our aim was to analyze the influence of two different treatment regimes in the development of behavioral sensitization measuring both enhanced rotational response and expression of more complex motor behaviors (AIMs) in an animal model of advanced PD. Our ultimate goal was to discriminate between drugs with different dyskinesia-inducing potential among the existing dopaminergic agents and those available in the future. A secondary aim was the evaluation of the long term effect of the apomorphine (APO) behavioral test (priming) on subsequent exposure to DA agonists using the same behavioral measurements. Finally, we measured c-*fos* immunoreactivity at the striatal level, in an attempt to find a molecular marker that would correlate with the behavioral observations, as there is evidence suggesting that c-*fos* expression could be implicated in the development of behavioral sensitization due to repeated treatment with dopamine agonists (APO, L-Dopa) [6,23].

2. Materials and methods

2.1. Animals

Female Wistar rats (205 ± 15 g) were caged in groups of three in a temperature-controlled room (21 °C) with a 12:12 h light/dark cycle (lights on at 8 a.m.), and ad libitum access to food pellets and tap water.

2.2. Drugs

Ketamine 50 mg/ml (Holliday-Scott), xylazine (Rompun, Bayer), desipramine hydrochloride (RBI, USA) and apomorphine chlorhydrate (APO-go 10 mg/ml, Medeva Pharma) and (−)quinpirole hydrochloride (RBI, USA) were dissolved in bidistilled water. 6-OHDA hydrobromide (RBI, USA) was dissolved in ascorbic acid (0.1%).

2.3. Surgical procedure

Rats were pretreated with 25 mg/kg (i.p.) of desipramine, 30–45 min before stereotaxic lesioning, to protect norepinephrine nerve terminals. Under deep surgical anesthesia (ketamine: 40 mg/kg; xylazine: 2 mg/kg i.p.), each rat received an injection of 6-OHDA (3.75 μg/μl, 4 μl/8 min, in order to produced a severe lesion) into the left medial forebrain bundle (MFB) (stereotaxic coordinates used: 2.8 mm posterior from Bregma; 2.0 mm lateral from Bregma; 8.6 mm ventral from Dura; tooth bar: −3.3 mm below the interaural line) [26].

2.4. Evaluation of the 6-OHDA lesion

Two weeks post-surgery, selection of the successfully denervated animals was performed by testing akinesia of the contralateral paw with a limb-use asymmetry test [29], to avoid drug administration while testing (see priming effect, [20]). Briefly, this test evaluates forelimb use during vertical exploration in a cylindrical enclosure. To perform this test, rats were put in a acrylic cylinder (20 cm diameter, 30 cm height) and an observer counted the number of wall contacts performed independently with the left or the right forepaw for 5 min (one session only). No habituation to the cylinder was allowed. Animals with more than 80% use of the ipsilateral paw were considered to be appropriately denervated and used for the experiment. In addition, tyrosine hydroxylase (TH) immunohistochemistry was performed at the end of the experiment in order to confirm the extent of the lesion (data not shown).

2.5. Chronic treatment with DA agonists and behavioral tests

Three weeks following surgery, animals were separated into three groups: the naive group (received two pretreatment injections with bidistillated water), the apomorphine (APO) primed group (received two pretreatment injections with APO at 6 days interval, 0.25 mg/kg, s.c.), and the quinpirole (QP) primed group (received two pretreatment injections with QP at 6 days interval, 0.1 mg/kg, i.p.). This pretreatment reproduced the sequence of pharmacological test that 6-OHDA-lesioned animals are often exposed to in order to test the degree of the lesion. The choice of doses of the DA agonists used in the experiments was based on a dose–response curve that showed that at the doses selected the animals developed the same degree of turning behavior (data not shown). Both APO and QP are drugs widely used in animal experiments of this kind and share similar pharmacodynamic and pharmacokinetic properties with those used in clinical practice.

Thirty days after pretreatment, a chronic administration treatment was performed (sensitization period). Each group was separated into three subgroups and treated with QP (0.1 mg/kg, i.p.), APO (0.25 mg/kg, s.c.) or vehicle (VEH,

Table 1
Summary of the groups of rats with unilateral 6-OHDA lesion used

Group	*N*	Pretreatment	Treatment
VEH/VEH	7	VEH	VEH
VEH/APO	12	VEH	APO
VEH/QP	12	VEH	QP
APO/VEH	7	APO	VEH
APO/APO	12	APO	APO
APO/QP	12	APO	QP
QP/VEH	7	QP	VEH
QP/APO	9	QP	APO
QP/QP	9	QP	QP

bidistillated water, i.p.), every 3 days for 3 weeks (8 injections in 21 days) (Table 1 and Fig. 1). We used this schedule in an attempt to maximize the sensitization effect [21]. Contralateral turning behavior was recorded in an automatic rotameter for 2 h after the injection, and an AIMs test was performed [16]. The Abnormal Involuntary Movements Scale (AIMS) is basically a test that quantifies three abnormal involuntary movements. These three AIMs were analyzed separately: forelimb dyskinesias (FD), axial dystonia (AD) and masticatory dyskinesias (MD), and rated on a scale that goes from 0 to 4 (0: absent; 1: occasional; 2: frequent; 3: continuous interrupted by sensory distraction; 4: continuous not interrupted by sensory distraction) [16]. Rats were assessed before injection of the DA agonists or VEH and every 30 min for 2 h (total = 5 measurements). Each measure was performed for 2 min by a blinded observer. The AIMs score was the sum of all measurements obtained from sequential assessments performed after the injection of the DA agonists. The theoretical maximum score that could be accumulated by one animal in one testing session (2 h) was 60, but only the peak dyskinesia activity was considered (maximum score per observation point: 12). The behavioral tests were performed between 11 a.m. and 5 p.m.

2.6. Inmunohistochemistry

2.6.1. TH immunostaining

Two hours after the last drug session, rats were deeply anaesthetized with Equitesin (3 ml/kg body weight, i.p.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS; 0.1 M, pH 7.4) followed by 4% paraformaldehyde in 0.1 M PBS (300 ml).

Animals were decapitated after perfusion. Brains were cryoprotected in sucrose gradient solutions (10, 15 and 20% in PBS, 0.1 M, pH 7.4), frozen in isopentane at −30 °C and stored at −60 °C until processing. Coronal, 40-μm-thick tissue sections were cut at −20 °C in a freezing microtome throughout the SNpc/VTA complex. The slices were stored in PBS containing 0.1% sodium azide at 4 °C until inmunohistochemistry was performed.

Immunohistochemical detection was performed on free-floating nigral sections (anterior–posterior (AP) coordinate from Bregma 3.7 mm, plate 39, [26]). After washing with PBS (0.1 M), tissue sections were treated in a blocking solution of 0.1 M PBS containing 2% BSA and 0.3% Triton X-100 (30 min) and incubated overnight at 4 °C with monoclonal mouse anti-TH (Boehringer), working dilution 1:1000. Three rinses in PBS were performed before incubating the sections with anti-mouse IgG (Amersham), working dilution 1:1000. The antibody–antigen complex was visualized by means of an avidin–biotin peroxidase complex (ABC) (Vector Laboratories), developed with 3,3′-diaminobenzidine and H_2O_2 in PBS (0.1 and 0.001%, respectively). The sections were mounted on gelatin-coated slides, dehydrated and coverslipped.

To examine the extent of dopaminergic denervation present in the substantia nigra, TH-immunoreactivity was evaluated on the lesioned and non-lesioned side. Only animals with a massive depletion of TH-positive neurons on the side ipsilateral to the lesion were included in this study.

2.6.2. c-Fos immunostaining

To stain for c-Fos immunoreactivity, criostat sections (20 μm) from the rostral striatum (corresponding to plates 13 and 14, coordinates from Bregma AP 1.2 and 1 mm, [26]) were performed with the basic immunohistochemistry protocol described above. Nickel chloride (0.08%) was added to the DAB solution to intensify the staining. The following antisera were used: polyclonal rabbit anti-c-Fos (Santa Cruz, USA), working solution 1:2000; anti-rabbit IgG (Vector), working solution 1:200.

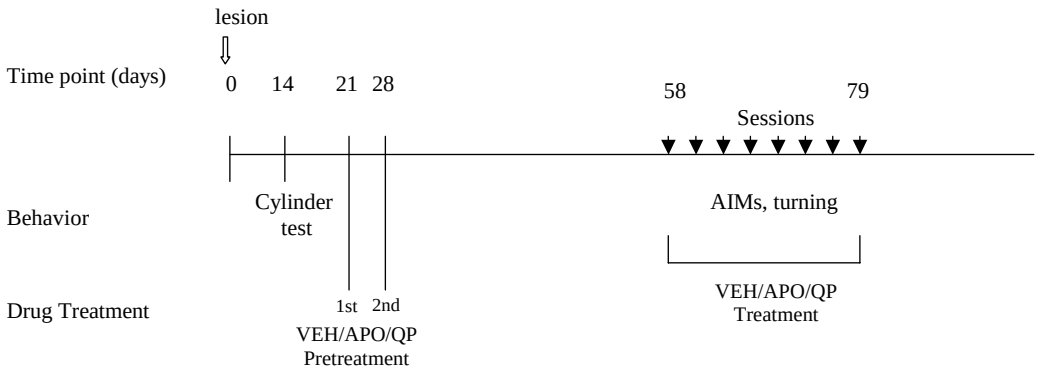


Fig. 1. Time course summarizing the order of drug administration and behavioral tests.

Controls for the specificity of primary antisera used were carried out by substitution of primary antibody by PBS.

For quantification of c-Fos, we analyzed the sections using a Scion Image Analyzer. A rectangular box with an area of 0.16 mm^2 was placed over the dorsolateral striatum and all immuno-positive cells were counted within that area. Data are expressed as the number of cells (mean ± S.E.M.) expressing c-*fos* in 0.16 mm^2. Photomicrographs of c-*fos* were obtained using the system described above.

2.7. Expression of the data and statistical analysis

Data used for the statistical analysis were expressed as: peak rotational activity (net turns per minute) and peak dyskinesia activity (score in a single AIMs evaluation time point, range 0–12). For data corresponding to each individual AIM the maximum possible score was 4. We chose the peak rotational and dyskinesia activity because of the different pharmacokinetic profiles of the drugs used in the experiments. This was done in order to obtain a normalized expression of the data.

Rotational behavioral and AIMs data were analyzed by means of an analysis of variance (ANOVA) with repeated measures, where groups (Table 1) were entered as the independent variable and the sessions as repeated measures. In all tests significance was assigned when $P < 0.05$. Post hoc comparisons were performed where appropriate using the Tukey test. A one-way ANOVA was performed on data of the first treatment session, to test for a possible delayed effect of the pretreatment. c-Fos was analyzed by one-way ANOVA where group was entered as the independent factor.

3. Results

3.1. Rotational behavior

DA agonists elicited rotational behavior contralateral to the lesion side in all animals in the course of sensitization. All groups (except VEH-treated group) showed a progressive enhancement of rotational behavior following the second treatment session. This is related to the development of sensitization induced by repeated exposure to DA agonists [3,7,8,10]. No differences between groups were found at the end of the treatments (Fig. 2A).

In the first session of the sensitization protocol, rats pretreated either with APO or QP exhibited robust contralateral rotation after a DA agonist challenge. Within the VEH pretreated rats, those receiving QP or VEH in the first session of the sensitization protocol showed no significant rotational behavior. In contrast VEH pretreated animals receiving APO in the first sensitization session displayed strong rotational behavior as reported before (Fig. 2B). This difference was statistically significant, and related to the priming phenomenon induced by previous exposure to DA agonists [10,28].

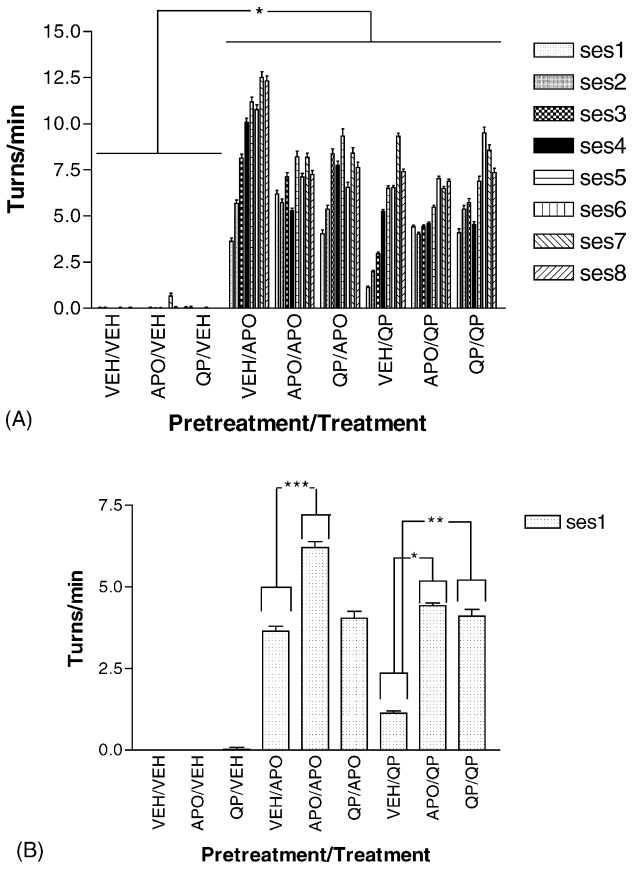


Fig. 2. (A) Peak rotational activity (mean ± S.E.M.) induced by DA agonists during the sensitization treatment. ANOVA of repeated measures showed significant differences between groups ($F_{8,76} = 14.66$, $P = 9.83^{-13}$) and between sessions ($F_{7,532} = 5.27$, $P = 7.82^{-6}$). Post hoc analysis between treatments showed significant differences between VEH- and APO-treated rats ($^{*}P < 2^{-3}$) and between VEH- and QP-treated rats ($^{*}P < 0.04$). No significant differences were found between APO- and QP-treated rats ($P > 0.08$). First session differed significantly from the rest sessions ($P < 1^{-3}$). (B) Peak rotational activity induced by DA agonists during the first session (effect of the pretreatment). ANOVA showed significant differences between groups ($F_{8,90} = 5.74$, $P = 6.61^{-6}$). Post hoc analysis showed significant differences between VEH/QP vs. APO/QP ($^{*}P = 4^{-3}$), VEH/QP vs. QP/QP ($^{**}P = 0.02$), APO/APO vs. VEH/APO ($^{***}P = 1.2^{-3}$).

3.2. AIM behavior

The sensitization treatment induced the development of AIMs, which were more pronounced in the animals treated with APO compared with QP-treated rats. VEH-treated animals showed almost no AIMs. Interestingly, the APO/QP group showed a higher degree of AIMs compared to the other QP (QP/QP and VEH/QP, $P = 0.06$ and 0.0001, respectively) treated animals but less than the APO (VEH/APO, APO/APO, and QP/APO) treated ones, suggesting the persistence of a stronger or longer lasting priming effect of APO. No significant differences were found within APO-treated rats ($P > 0.5$) (Fig. 3A).

APO-treated animals did not show differences irrespective of the pretreatment received, and displayed a high degree

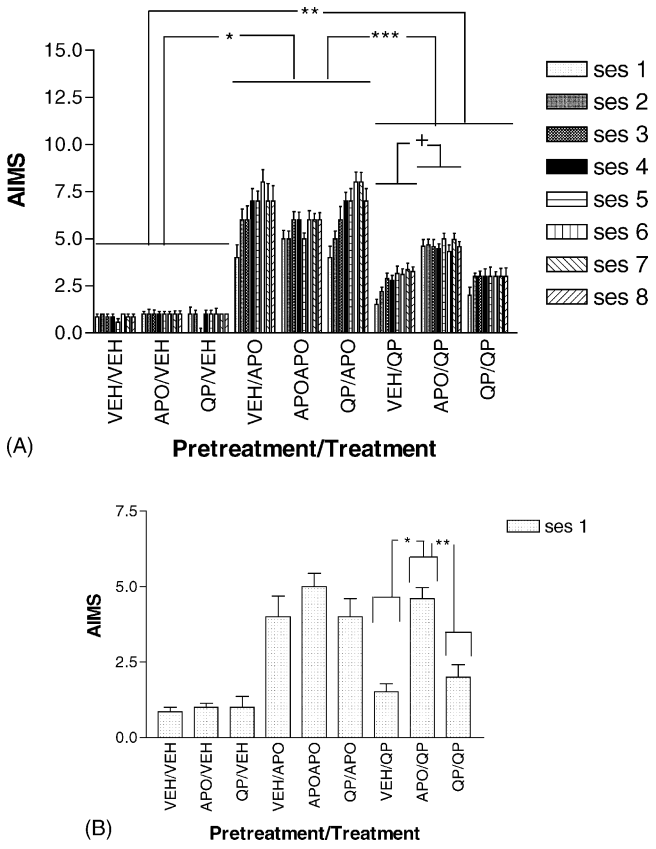


Fig. 3. (A) Peak dyskinesia activity (mean ± S.E.M.) induced by DA agonists during the sensitization treatment. An ANOVA test of repeated measures of total AIMs for all sessions was performed showing significant differences between groups ($F_{8,90} = 43.93$, $P = 7.37^{-28}$), between sessions ($F_{7,630} = 10.98$, $P = 3.97^{-13}$) and the interaction ($F_{56,630} = 2.76$, $P = 9.69^{-10}$). Post hoc analysis between groups showed significant differences between VEH- and APO-treated rats ($^{*}P < 1^{-4}$) and between VEH- and QP-treated rats ($^{**}P < 0.02$). Also significant differences between APO-treated rats and between QP-treated rats were seen ($^{***}P < 7^{-3}$), except APO/QP vs. APO/APO ($P = 0.27$). Within QP-treated rats significant differences were seen only between VEH/QP and APO/QP ($^{+}P = 1^{-4}$). Within APO-treated rats no significant differences were found ($P > 0.5$). (B) Peak dyskinesia activity during the first session. A one-way ANOVA was performed showing significant differences between groups ($F_{8,90} = 14.29$, $P = 3.01^{-13}$). Post hoc analysis showed significant differences between VEH/QP and APO/QP ($^{*}P = 2^{-7}$), QP/QP vs. APO/QP ($^{**}P = 6^{-3}$).

of AIMs already in the first session of the sensitization protocol. Within the QP-treated animals, only those pretreated with APO exhibited a significant level of AIMs during the first sensitization session. Indeed, this group did not differ significantly from those treated with APO, while VEH/QP and QP/QP behaved similarly to VEH-treated animals (Fig. 3B).

3.3. Forelimb dyskinesia, axial dystonia and masticatory dyskinesia

Although significant differences were observed between the different regimes when measuring total AIMs, an analysis of each individual type of AIM was performed in order to study their relative contribution to this behavior.

APO-treated animals developed higher levels of FD compared with QP- or VEH-treated rats (Fig. 4A). The APO/QP group showed an intermediate behavior between APO- and QP-treated rats. In the first sensitization session, animals pretreated with APO and subsequently challenged with APO were the ones displaying the highest degree of FD, followed by VEH/APO, APO/QP, and QP/APO. VEH/QP and QP/QP groups did not differ significantly from VEH-treated animals (Fig. 4A′).

AD showed a similar behavior to FD, with APO-treated animals developing higher levels than QP- or VEH-treated ones. Within the QP-treated groups, APO pretreated rats showed a significantly higher score than those pretreated with VEH (Fig. 4B). In the first sensitization session APO/QP animals had AD scores as high as APO/APO, QP/APO, and VEH/APO. We also observed significant differences between APO/QP and VEH/QP or QP/QP pretreated groups (Fig. 4B′).

Evaluation of MD showed significant differences between VEH-treated animals and the remaining groups. We did not find differences between APO- and QP-treated rats at the end of the treatment period (Fig. 4C). In the first session, however, we found significant differences between VEH/QP and APO/QP groups (Fig. 4C′).

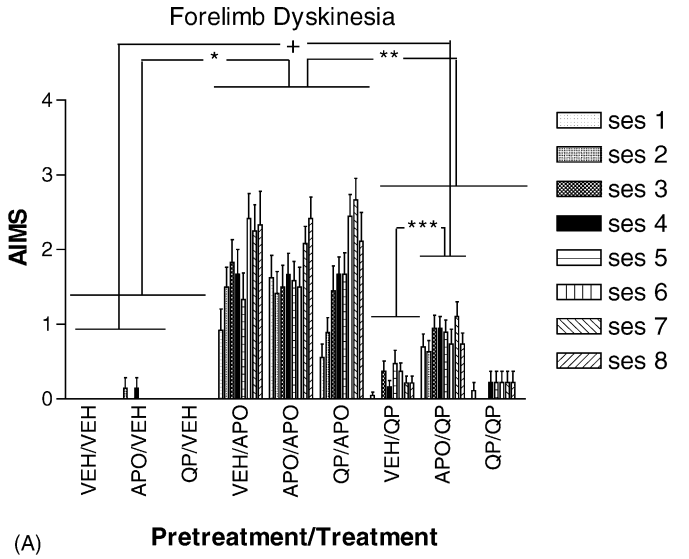
Our results indicate that FD and AD are both the main contributing factors and can better differentiate between treatments. VEH-treated rats showed almost no AIMs.

3.4. Expression of c-Fos in the lesioned striatum

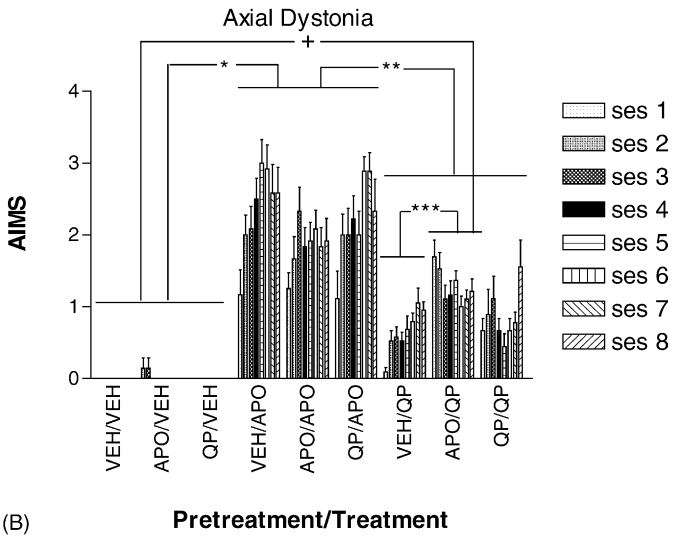
Analysis of c-Fos was performed only in the lesioned striatum as no immunodetectable c-Fos cells were found in the striatum contralateral to the lesion. All APO-treated groups expressed c-Fos immunoreactivity, compared to VEH- or QP-treated animals in which c-Fos was non-detectable (Fig. 5). No differences were found within the APO treatment groups (VEH/APO, QP/APO, APO/APO) ($P > 0.42$), indicating that the ability of repeated injections of APO to induce c-*fos* expression in the lesioned striatum was not influenced by pretreatment (Table 2).

4. Discussion

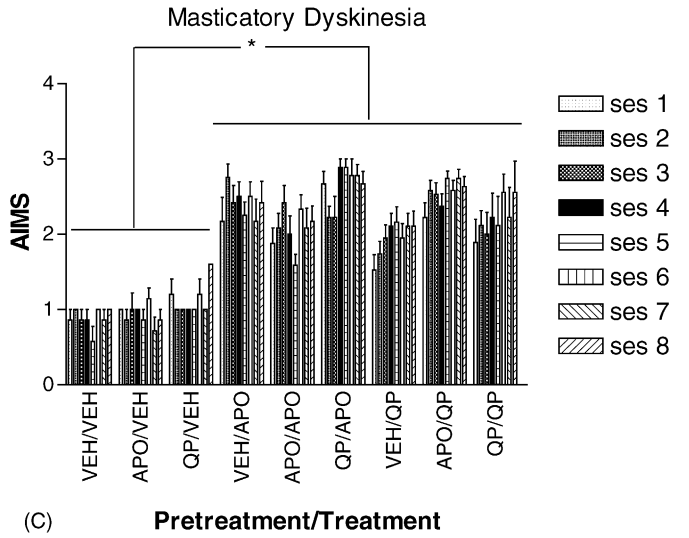
Despite the widespread use of the 6-OHDA-lesioned rat model to explore the effects and mechanism of action of antiparkinsonian drugs, some skepticism has been expressed about its validity in modeling parkinsonian symptoms and treatment-related dyskinesias [24]. It has been suggested that only primates may be physically capable of showing the spectrum of movement disorders which are displayed by patients [4]. However, in recent studies, it has become apparent that rats can perform more complex and articulate


Forelimb Dyskinesia
AIMS
ses 1
ses 2
ses 3
ses 4
ses 5
ses 6
ses 7
ses 8
VEH/VEH
APO/VEH
QP/VEH
VEH/APO
APO/APO
QP/APO
VEH/QP
APO/QP
QP/QP
Pretreatment/Treatment

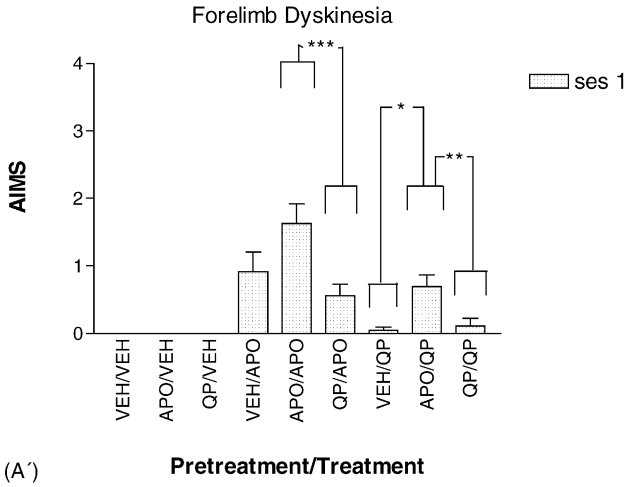
(A)


Axial Dystonia
AIMS
ses 1
ses 2
ses 3
ses 4
ses 5
ses 6
ses 7
ses 8
VEH/VEH
APO/VEH
QP/VEH
VEH/APO
APO/APO
QP/APO
VEH/QP
APO/QP
QP/QP
Pretreatment/Treatment

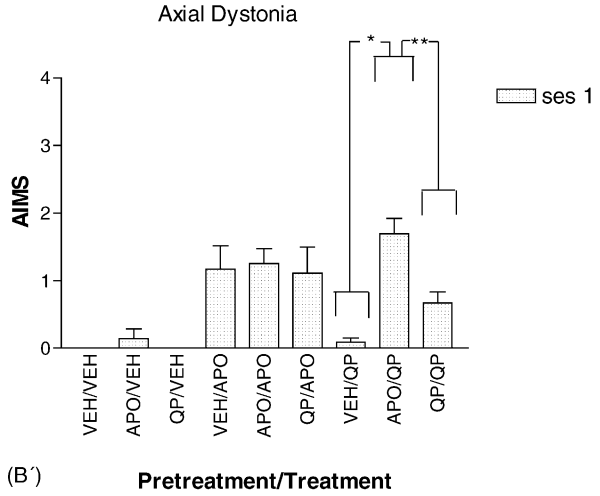
(B)


Masticatory Dyskinesia
AIMS
ses 1
ses 2
ses 3
ses 4
ses 5
ses 6
ses 7
ses 8
VEH/VEH
APO/VEH
QP/VEH
VEH/APO
APO/APO
QP/APO
VEH/QP
APO/QP
QP/QP
Pretreatment/Treatment

(C)


Forelimb Dyskinesia
AIMS
ses 1
VEH/VEH
APO/VEH
QP/VEH
VEH/APO
APO/APO
QP/APO
VEH/QP
APO/QP
QP/QP
Pretreatment/Treatment

(A´)


Axial Dystonia
AIMS
ses 1
VEH/VEH
APO/VEH
QP/VEH
VEH/APO
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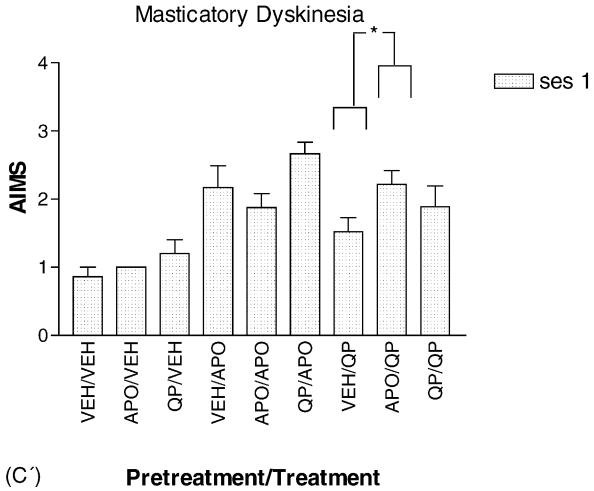
QP/APO
VEH/QP
APO/QP
QP/QP
Pretreatment/Treatment

(B´)


Masticatory Dyskinesia
AIMS
ses 1
VEH/VEH
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QP/QP
Pretreatment/Treatment

(C´)

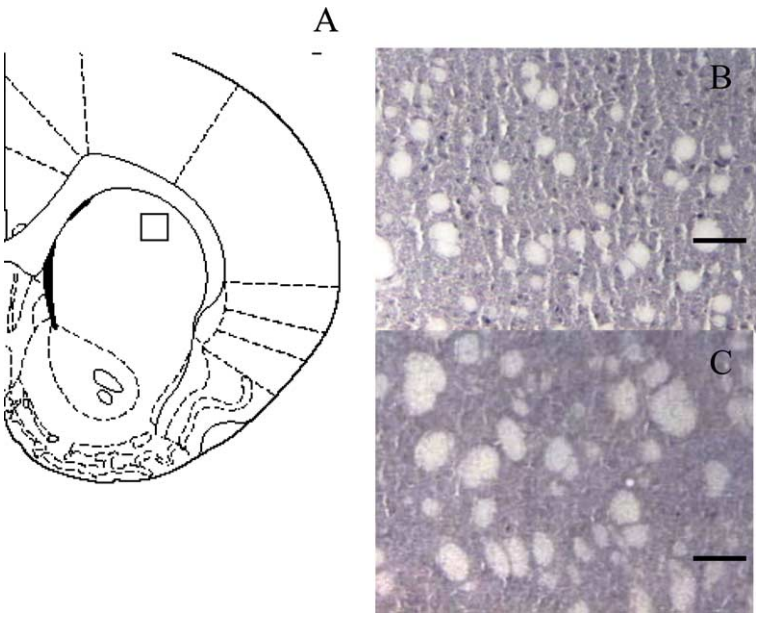

Fig. 5. The number of c-*fos* inmunoreactive cells counted in the dorsolateral striatum (area sampled: 0.16 mm^2). (A) Schematic representation of the striatal region where c-*fos* has been quantified. (B) Photomicrograph showing c-*fos* expression in the lesioned side of the striatum of a representative QP/APO-treated animal. (C) Photomicrograph showing transverse section of the ipsilateral striatum of a representative of QP/QP-treated animal. Note the complete absense of inmunoractive c-*fos* cells. Scale bar: 100 μm.

Table 2
Data showing number of expressing immunoreactive c-*fos* cells (mean ± S.E.M.) in an area of 0.16 mm^2 of the treated groups

Groups	c-*fos* (mean ± S.E.M.)
VEH/VEH	ND
VEH/QP	ND
VEH/APO	157.5 ± 56.3
QP/VEH	ND
QP/QP	ND
QP/APO	136 ± 22.47
APO/VEH	ND
APO/QP	ND
APO/APO	136 ± 25.98

ND: non-detectable.

behaviors, and a wide range of tests have been proposed to assess motor function in parkinsonian rats [9,16,29,35]. On this issue there is an ongoing controversy, as there is little agreement as to which behavioral test in rats would provide measures that can predict motor complications and clinical benefits. Many studies have considered rotational behavior as an antiparkinsonian effect, and its enhancement through repeated exposure to a dopaminergic agent an equivalent to levodopa-induced motor complications [14,30,32,33]. Thus far, drug-induced rotation has constituted the standard measure of behavioral outcome in unilaterally 6-OHDA-lesioned rats and has been used to model both parkinsonian disability and dyskinetic effects of drug treatments [19]. In recent years, several groups highlighted the nonspecificity of

Fig. 4. Peak dyskinesia activity of each AIM (mean ± S.E.M.). (A) Forelimb diskinesia (FD): ANOVA showed significant differences between groups ($F_{8,90} = 29.30$, $P = 5.83^{-22}$), between sessions ($F_{7,630} = 7.17$, $P = 2.81^{-8}$) and in the interaction ($F_{56,630} = 2.90$, $P = 1.35^{-10}$). Post hoc analysis between groups showed no differences between VEH- and QP-treated animals, except APO/QP (+) (vs. VEH/VEH ($P = 0.03$), vs. APO/VEH ($P = 0.04$)). No differences were found within VEH/QP and QP/QP, and QP/QP and APO/QP, significant differences between VEH/QP and APO/QP were seen ($^{***}P = 3^{-3}$). No differences within APO-treated animals were found. Significant differences were found between APO- and QP-treated animals ($^{**}P = 8^{-3}$) and between APO- and VEH-treated group ($^{*}P = 1^{-4}$). Post hoc analysis between sessions showed significant differences between the first session and the rest of the sessions ($P < 2^{-3}$). (B) Axial dystonia (AD): ANOVA showed significant differences between groups ($F_{8,90} = 25.55$, $P = 4.08^{-20}$), between sessions ($F_{7,630} = 6.40$, $P = 2.73^{-7}$) and the interaction ($F_{56,630} = 3.05$, $P = 1.37^{-11}$). Post hoc analysis between groups showed no differences between VEH- and QP-treated animals, except APO/QP (+) (APO/QP vs. VEH/VEH ($P = 2^{-3}$), vs. QP/VEH ($P = 0.01$), vs. APO/VEH ($P = 3^{-3}$)). No differences were found within VEH/QP and QP/QP, and QP/QP and APO/QP, significant differences between VEH/QP and APO/QP were seen ($^{***}P = 0.03$). No differences within APO-treated animals were found. Significant differences were found between APO-treated and VEH ($^{*}P < 1^{-4}$) and between APO- and QP-treated animals ($^{**}P < 0.01$), except APO/QP vs. APO/APO. Post hoc analysis between sessions showed significant differences between the first session and the rest of the sessions ($P < 4^{-3}$). (C) Masticatory diskinesia (MD): ANOVA showed significant differences between groups ($F_{8,88} = 18.76$, $P = 4.03^{-16}$), and between sessions ($F_{7,616} = 2.11$, $P = 0.04$). Post hoc analysis between groups showed significant differences between vehicle-treated rats and the rest of the APO and QP treatments ($^{*}P < 0.03$). (A′) FD: ANOVA for the first session was significant ($F_{8,102} = 9.67$, $P = 6.75^{-10}$). Post hoc analysis showed significant differences between VEH/QP vs. APO/QP ($^{*}P = 1^{-3}$); APO/QP vs. QP/QP ($^{**}P = 0.02$); APO/APO vs. QP/APO ($^{***}P = 1.52^{-5}$). (B′) AD: ANOVA for the first session was significant ($F_{8,102} = 8.73$, $P = 5.02^{-9}$). Post hoc analysis showed significant differences between VEH/QP vs. APO/QP ($^{*}P = 6.36^{-10}$); APO/QP vs. QP/QP ($^{**}P = 1^{-3}$). (C′) MD: ANOVA for the first session was significant ($F_{8,102} = 3.94$, $P = 4^{-4}$). Post hoc analysis showed significant differences between VEH/QP vs. APO/QP ($^{*}P = 6^{-3}$).

rotation as a measure of behavioral outcome in the parkinsonian rat model and provided evidences that this behavior does not parallel an improvement in physiological motor function. Behavioral sensitization (enhancement of rotational behavior) is indeed observed independently of the drug regime used. Several studies have shown that daily injections of either L-Dopa or QP alone led to an enhanced behavioral responsivity [3,27]. Moreover, there have been no studies addressing the long-term effects that previous exposure to APO used in the selection of well denervated animals produces in following behavioral studies.

In our study we were able to demonstrate the strong effects produced by previous exposure to a DA agonist (priming) on subsequent behavioral evaluations. We found that two exposures to a DA agonist, 1 month before the sensitization treatment began, induced in all animals a high degree of turning behavior compared to the VEH pretreated groups. This is in agreement with previous reports [27]. A novel finding of this study is related to the effect of priming on dyskinesias. Interestingly enough, within the QP-treated animal groups, only those previously exposed to APO showed a significant degree of AIMs on the first treatment session. Moreover there appeared to be a quite robust carry-over effect as these animals continued to show a higher degree of AIMs during the entire length of the treatment period in comparison to those previously exposed to either VEH or QP, although never reaching the degree observed in the APO-treated animals. Although QP has been previously found to be able to induce dyskinesias in parkinsonian monkeys, this was only observed after the animals had been exposed to L-Dopa [13]. The rotational behavior of naive (VEH pretreated) and primed (APO or QP pretreated) animals on the first subsequent exposure to a DA agonist at the beginning of the treatment period clearly showed that contralateral turns and AIMs behave significantly different [16]. All this evidence taken together supports the notion that previous exposure to DA agonists (APO) for the selection of well lesioned animals can influence the outcome of subsequent experiments [10,20]. Furthermore, the finding that a previous exposure to a DA agonist like APO has such a pervasive effect on rotation and dyskinesias underlines the importance of the priming phenomenon, and the need to bear it in mind in the clinical setting.

Secondly, we were able to show that the use of these behavioral tools allowed us to discriminate between dopaminergic drugs with different stimulation profiles and pharmacokinetic properties. We could confirm previous results [17] that rotational behavior and dyskinesias (AIMs) proceed in parallel or not depending on the type of DA agonist used to sensitize the animals. In the APO sensitized animals there is indeed a parallel enhancement of both behavioral outcome measures. On the other hand, the QP sensitized animals showed a dissociated behavior, with significant enhancement of rotational behavior similar to what is observed in APO sensitized rats, but with significantly less induction of dyskinesias. Measurement of total AIMs was found to be significantly different in APO- and QP-treated animal groups. Moreover, a separate analysis of the three different types of AIMs (MD, AD, and FD) observed in this model provided useful and additional information, and strengthened the differences observed between the APO- and QP-treated animals. These findings suggest that FD and AD can be good indicators of a differential behavioral response identifying those animals treated with APO from those treated with QP. In our hands results of MD evaluation did not have the same discriminating power as to the type of drug regime the animals had been exposed to. This is in contradiction with recent publications [17]. However, we are aware that this could have been the result of an observer bias, as in some cases, normal orolingual movements and abnormal MD can be confused, leading to an overestimation of MD values. A differential analysis of AIMs would be in our view the best way to overcome this confounding factor, and more properly evaluate the dyskinesia-inducing potential of dopaminergic drugs in rats.

Although the drugs used in the present study and the administration regime employed are not the same as those used in clinical practice, both APO and QP have pharmacokinetic and pharmacodynamic profiles that closely resemble those of levodopa and the routinely used selective D2 DA agonists. In support of this observation we have preliminary results showing that 6-OHDA lesioned rats treated for 1 month with either L-Dopa or Pramipexole, administered orally on a daily basis, developed increased number of rotations without significant differences between both groups, but with a clearly distinct dyskinetic behavior. Pramipexole-treated rats developed significantly less dyskinesia despite showing equivalent enhancement of rotational behavior as L-Dopa (data not shown).

The expression of the immediate-early gene c-*fos* is considered a marker of neural activation. L-Dopa, cocaine and other DA agonists induce the expression of c-*fos* in the striatum ipsilateral to the lesion, most likely through mechanisms that reflect postsynaptic DA D1 or D1/D2 receptor stimulation [3,12,15,22,23,25]. Although the c-*fos* response has been shown to desensitize after repeated challenge with either D1 or mixed D1/D2 agonists, animals exposed to these drugs still retain a significant degree of immunoreactivity compared to those given vehicle or D2 agonists in which no immunoreactivity is observed [3, this paper]. Our study showed the ability of APO to induce c-*fos* in DA denervated striatum, while no c-*fos* expressing cells were detected in QP- or VEH-treated animals. This is consistent with recent findings in normal rats that showed that QP inhibits c-*fos* induction by a selective D1 receptor agonist in neurons of the islands of Calleja, which contain D1 and D3 receptors [31]. Interestingly enough, all APO-treated animals developed high levels of dyskinesia in contrast to QP-treated rats. The only exception being those animals that were primed with APO and subsequently treated with QP, in whom, in the absence of c-*fos* expression, a high level of dyskinesia was observed. A likely explanation to this

apparently paradoxical finding could be (a) that with this particular treatment regime, induction of c-*fos* expression is a transient phenomenon, turned-off by chronic QP treatment, setting in motion a cascade of events that persist beyond the window in time in which the gene is detected and relates to the subsequent development of dyskinesias, or (b) that c-*fos* expression and dyskinesias are two unrelated phenomena. Recent studies suggest that FosB/ΔFosB would be better markers for dyskinesia as they do not undergo desensitization after chronic exposure to DA agonists [1,2,35]. However, c-*fos*, as a non-specific marker of neuronal activation, was still able to discriminate between animals chronically treated with APO versus those treated with QP [6,23].

Our results cannot explain the mechanisms leading to an enhancement of the rotational behavior observed in APO- or QP-treated animals, nor can they provide an insight into the reasons why APO-treated animals were the only ones that developed significantly higher levels of dyskinesia. However, we believe we have demonstrated that it is possible to differentiate drugs with dissimilar dyskinesia-inducing potential through this simple behavioral paradigm in rodents. Moreover, we were able to show that, depending on the type of DA agonist, dyskinesias and rotational behavior do not necessarily run a parallel course of enhancement, a finding that would suggest that these two behaviors may be the result of functional modifications at different output pathways controlling motor activity. The observation that chronic APO treatment can reset peak-dose dyskinesia threshold in L-Dopa-treated patients could be used as an argument against our findings, however in the clinical setting, APO was given continuously [18], while in our case it was administered in a pulsatile form. Additional studies should be made testing the two principal hypotheses: overstimulation of the D1 DA receptor and the short duration of action (pulsatile-like stimulation of DA receptors) of a given DA agonist [5].

In conclusion with these results we have confirmed previous studies showing that it is possible to observe involuntary movements resembling LID in 6-OHDA lesioned rats [16,17,35], and that these quali-quantitative aspects of the motor response are better suited to provide information on the phenomenon of drug induced dyskinesias than the degree of rotational enhancement. Furthermore, we believe this paradigm could be routinely applied to the evaluation of different DA agonists with high or low propensity to induce dyskinesias.

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