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Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease

M. Lundblad,[1] M. Andersson,[1] C. Winkler,[1,2] D. Kirik,[1] N. Wierup[1] and M. A. Cenci[1]
[1]Department of Physiological Sciences, Lund University, Wallenberg Neuroscience Centre, Neurobiology Division, BMC A11, S-221 84 Lund, Sweden
[2]Department of Neurology, Hannover Medical School,Hannover, Germany

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Abstract

In an attempt to define clinically relevant models of akinesia and dyskinesia in 6-hydroxydopamine (6-OHDA)-lesioned rats, we have examined the effects of drugs with high (L-DOPA) vs. low (bromocriptine) dyskinesiogenic potential in Parkinson's disease on three types of motor performance, namely: (i) abnormal involuntary movements (AIMs) (ii) rotational behaviour, and (iii) spontaneous forelimb use (cylinder test). Rats with unilateral 6-OHDA lesions received single daily i.p. injections of L-DOPA or bromocriptine at therapeutic doses. During 3 weeks of treatment, L-DOPA but not bromocriptine induced increasingly severe AIMs affecting the limb, trunk and orofacial region. Rotational behaviour was induced to a much higher extent by bromocriptine than L-DOPA. In the cylinder test, the two drugs initially improved the performance of the parkinsonian limb to a similar extent. However, L-DOPA-treated animals showed declining levels of performance in this test because the drug-induced AIMs interfered with physiological limb use, and gradually replaced all normal motor activities. L-DOPA-induced axial, limb and orolingual AIM scores were significantly reduced by the acute administration of compounds that have antidyskinetic efficacy in parkinsonian patients and/or nonhuman primates (–91%, yohimbine 10 mg/kg; –19%, naloxone 4–8 mg/kg; –37%, 5-methoxy 5-N,N-dimethyl-tryptamine 2 mg/kg; –30%, clozapine 8 mg/kg; –50%, amantadine 40 mg/kg). L-DOPA-induced rotation was, however, not affected. The present results demonstrate that 6-OHDA-lesioned rats do exhibit motor deficits that share essential functional similarities with parkinsonian akinesia or dyskinesia. Such deficits can be quantified using novel and relatively simple testing procedures, whereas rotometry cannot discriminate between dyskinetic and antiakinetic effects of antiparkinsonian treatments.

Introduction

Since its first description (Ungerstedt, 1968), the 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in the rat has provided an invaluable tool for investigating the pathophysiology of dopamine (DA) denervation and for evaluating novel therapeutical options (for review see Schwarting & Huston, 1996). The feasibility of radically new treatment stategies for Parkinson's disease, e.g. brain transplants (for review see Herman & Abrous, 1994; Lindvall, 1997) and gene therapy (Mandel *et al*., 1997; 1998; Choi-Lundberg *et al*., 1998; Kirik *et al*., 2000; Kordower *et al*., 2000) has been primarily assessed in this model.

Despite the widespread use of 6-OHDA-lesioned rats, some scepticism has been expressed about modelling parkinsonian symptoms and treatment-related dyskinesias in rodents (see, e.g., Nutt, 1990). It has been suggested that only primates may be physically capable of showing the repertoire of movement disorders which are displayed by patients (Bézard *et al*., 2001). Others have pointed out that the most critical factor in assuring predictive validity of animal models may not necessarily be the choice of species so much as the selection of behavioural measures (Schallert, 1995; Lindner *et al*., 1996). Thus far, drug-induced rotation has constituted the standard measure of behavioural outcome in unilaterally 6-OHDA-lesioned rats, and has been used to model both parkisonian disability and antiakinetic or dyskinetic effects of drug treatments (partially reviewed in Schwarting & Huston, 1996; see also Henry *et al*., 1998; Carey, 1991a, b). In recent years, it has become apparent that rats are indeed open to a more complex and articulate behavioural analysis, and a large variety of tests have been proposed to assess motor function in this species (see, e.g. Montoya *et al*., 1991; Olsson *et al*., 1995; Lindner *et al*., 1996; Rozas *et al*., 1997; Cenci *et al*., 1998; Lee *et al*., 2000; Schallert *et al*., 2000). However, most of these novel tests have not been fully characterized with respect to their sensitivity and response to antiparkinsonian compounds. Moreover, little effort has been devoted to defining potential advantages of novel testing paradigms over traditional rotometry.

In an attempt to shed light on the usefulness and limitations of different tests, we have carried out a pharmacological characterization of three measures of motor performance in 6-OHDA-lesioned rats, namely: (i) ratings of abnormal involuntary movements (AIMs; Cenci *et al*., 1998; Lee *et al*., 2000); (ii) automated recordings of rotation; and (iii) assessments of spontaneous forelimb use in the context of a physiological explorative response (cylinder test; Schallert & Tillerson 2000). We first analysed the response of these behavioural measures to the effects of L-DOPA or bromocriptine. The

Correspondence: Dr Angela Cenci Nilsson, as above.
E-mail: Angela.Cenci_Nilsson@mphy.lu.se

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latter compound is the prototype of a class of antiparkinsonian drugs which do not induce dyskinesia when administered de novo (Rascol *et al*., 1979; Lees & Stern, 1981; Bédard *et al*., 1986; Pearce *et al*., 1998; Rascol *et al*., 2000). We then tested five compounds which have a proven antidyskinetic efficacy in patients and/or nonhuman primate models of Parkinson's disease.

Materials and methods

Subjects

A total of 69 female Sprague-Dawley rats (B & K Universal, Stockholm, Sweden; ≈225 g body weight when purchased) were used in the study. They were housed under 12-h light : 12-h dark conditions with *ad libitum* access to food and water. The treatment of the animals and their conditions were in accordance with internationally accepted guidelines, and had been approved by local authorities (permit no. 198–99 issued by Malmö-Lund Ethical Committee on Animal Research).

Grouping of the animals and experimental design

Pharmacodynamic characterization of L-DOPA and bromocriptine

This preliminary experiment comprised 8 intact rats and 25 6-OHDA-lesioned rats which were not used in the chronic drug treatment study later on.

Effects of chronic treatment with L-DOPA, bromocriptine or vehicle

This study comprised 36 rats with 6-OHDA injections in either the striatum (n = 18) or the medial forebrain bundle (n = 18). Animals were allotted to three groups which were matched with respect to both lesion type and amphetamine-induced rotational scores. At ≈6 weeks post lesion, the groups started to receive chronic treatment with either L-DOPA (n = 14), bromocriptine (n = 14) or vehicle (n = 8) for 20 days. During this period, recordings of AIMs and rotation were carried out on consecutive days twice a week, whilst the cylinder test was carried out once a week.

Acute challenge with antidyskinetic compounds

In order to test the ability of nondopaminergic compounds to modulate the effects of L-DOPA or bromocriptine we selected seven rats from each of the bromocriptine and L-DOPA groups which had developed appreciable AIMs (severity grade >1) or rotation during the chronic drug treatment period. An additional seven rats from the vehicle group were kept in the study to serve as controls. Rats were kept on a maintainance regimen consisting of 2–4 injections per week of the previously administered drug, as this administration frequency is sufficient to maintain stable AIM scores (Lee *et al*., 2000). Rats from the vehicle group were injected with physiological saline according to the same administration frequency. Each nondopaminergic compound was first tested for its ability to affect L-DOPA- and/or bromocriptine-induced AIMs. Animals were treated with the compound or its corresponding vehicle at a suitable interval after the injection of bromocriptine or L-DOPA (see below). In order to keep the behavioural investigator blind to the rats' individual treatment, the nondopaminergic drug was given randomly on either day 1 or day 2 of a two-consecutive-days testing period. Doses of nondopaminergic drugs that produced a significant reduction of drug-induced AIM scores were subsequently tested for their effects on limb akinesia (cylinder test) and rotation, as assessed using the same two-consecutive-days design as for the dyskinesia test. A time lag of 1–2 weeks was allowed between the testing of different drugs.

6-OHDA-lesions and behavioural screening

DA-denervating lesions were performed by unilateral injection of 6-OHDA into either the medial forebrain bundle (MFB) or the striatum. Injection of 6-OHDA in the MFB, at the origin of the nigrostriatal pathway, is the lesion procedure used in most studies (for review see Schwarting & Huston, 1996). Injection of 6-OHDA in the striatum has recently been shown to provide a more progressive model of DA-neuron degeneration, which is particularly useful for an assessment of neuroprotective treatments (Sauer & Oertel, 1994; Lee *et al*., 1996; Kirik *et al*., 1998; 2000).

Rats were anaesthetized with Equithesin (3 mL/kg, i.p.) and mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). 6-OHDA-HCl (Sigma-Aldrich Sweden AB) was dissolved in 0.02% ascorbate–saline at a concentration of 3 or 3.5 μg free base 6-OHDA per μL, in the case of MFB or intrastriatal lesions, respectively. Injections were performed at the rate of 1 μL/min (allowing an additional 3 min before retracting the needle) using a 10-μL Hamilton microsyringe with a 26-gauge steel cannula. For MFB lesions, 6-OHDA was injected into the right ascending DA fibre bundle at the following coordinates (in mm) relative to bregma and the dural surface: (1) A –4.4, L 1.2, V 7.8, tooth bar –2.4 (7.5 μg); (2) A –4.0, L 0.75, V 8.0, tooth bar +3.4 (6 μg). Intrastriatal 6-OHDA lesions were performed according to Kirik *et al*. (1998). Three 7-μg deposits of 6-OHDA were injected in the lateral caudate–putamen (CPu) at the following coordinates (in mm relative to bregma and the dural surface, tooth bar at 0.0): (1) A +1.0, L –3.0, V –5.0; (2): A –0.1, L –3.7, V –5.0; (3): A –1.2, L –4.5, V –5.0. In order to assess the efficacy of the lesions, all rats were tested for amphetamine-induced rotation at 2 weeks after the 6-OHDA injections. The animals' turning behaviour was recorded in an automated rotometer over a 90-min period after the intraperitoneal injection of 2.5 mg/kg dexamphetamine sulphate (Apoteksbolaget, Sweden), dissolved in saline. Rats showing rotational scores >4 net full turns/min in the direction ipsilateral to the lesion were kept in the study. These rotational scores were found to correspond to >90% depletion of specific [^{3}H]mazindol binding sites in the lateral (sensorimotor) caudate-putamen (data not shown).

Drugs and treatment regimens

All drugs were dissolved in a volume of 1 mL vehicle per kg; vehicle consisted of physiological saline solution unless otherwise stated. L-DOPA methyl ester (Sigma Chemicals Co., St. Louis, MO, USA) was given at a fixed dose of 6 mg/kg/day i.p., combined with 15 mg/kg/day of the DOPA-decarboxylase inhibitor benserazide–HCl (gift of Hoffman-LaRoche, Switzerland). Chronic treatment with this dose of L-DOPA has previously been shown to induce a gradual development of dyskinetic-like movements in 6-OHDA-lesioned rats (Andersson *et al*., 1999; Johansson *et al*., 2001; C. Winkler, D. Kirk, A. Björklund and M. A. Cenci, unpublished observations). Bromocriptine (2-bromo-α-ergocryptine) was either purchased from Sigma-Aldrich or kindly provided by Novartis Pharma AG (Switzerland). The drug was mixed with an equal weight of tartaric acid and two drops (90 μL) of 99% ethanol, subsequently dissolved in bi-distilled water, and given i.p (Silverman, 1992). The doses of bromocriptine tested in this study ranged between 1.25 and 5 mg/kg/day. A daily dose of 3.5 mg/kg bromocriptine was chosen for the chronic treatment experiment. Naloxone hydrochloride (purchased from Sigma-Aldrich Sweden AB) was given s.c. at doses ranging from 0.125 to 8 mg/kg (Carey, 1991a; Söderpalm & Svensson, 1999). Yohimbine hydrochloride (purchased from Sigma-Aldrich Sweden AB) was mixed with 45 μL of 20% acetic acid, and dissolved in 38-

°C bi-distilled water, according to the manufacturers' instructions. The drug was given i.p. at 1 and 10 mg/kg (Henry *et al*., 1998). 5-methoxy 5-N, N-dimethyl-tryptamine (5-MDOT; Sigma-Aldrich Sweden AB) was first pulverized with a pipette tip, then mixed with 45 μL of 1% acetic acid and 90 μL of 99% ethanol, and finally dissolved in 38-°C bi-distilled water, according to the manufacturers' instructions. The drug was given i.p at a dose of 2 mg/kg (Henry *et al*., 1998). Amantadine hydrochloride (Sigma-Aldrich Sweden AB) was given s.c (Blanchet *et al*., 1998) at a dose of 40 mg/kg. All the nondopaminergic compounds but amantadine were given 5 min after the injection of L-DOPA, and 100–105 min after the injection of bromocriptine. Due to its slow and prolonged pharmacokinetics (Menon *et al*., 1973; data not shown), amantadine was given simultaneously with bromocriptine and 100 min prior to L-DOPA.

TABLE 1. Post-injection intervals chosen for the recording of different behavioural tests

	Post-injection intervals (min)		
	AIM rating	Rotation	Cylinder test
L-DOPA	20–180	5–185	45–50
Bromocriptine	120–280	120–300	120–125
Vehicle	20–180	5–185	45–50

The animals' motor performances were recorded at postinjection intervals which matched the pharmacodynamic profile of the administered drugs (injections were made at 0 min). In the last part of the study, all nondopaminergic compounds (but amantadine) or their corresponding vehicles were given 5 min after the injection of L-DOPA, and 100–105 min after the injection of bromocriptine.

AIM recordings

Recordings of dyskinesia were performed from between 10.00 h and 16.00 h by an investigator who was kept completely unaware of the rats' group membership and pharmacological treatment (experimentally blind). For quantification of drug-induced abnormal involuntary movements (AIMs), rats were observed individually every 20th minute at 20–180 min after the injection of L-DOPA or vehicle, and 120–280 min after the injection of bromocriptine (Table 1). These different time lags match the pharmacodynamic profiles of the injected drugs (see Results). As AIMs were found to return to baseline by 140 min post L-DOPA-injection (see, e.g., Fig. 7A and B), a testing time of 160 min was used in the second part of the study. As described previously (Cenci *et al*., 1998; Andersson *et al*., 1999; Johansson *et al*., 2001), rat AIMs were classified into four subtypes: axial AIMs, i.e. dystonic posturing or choreiform twisting of the neck and upper body towards the side contralateral to the lesion; limb AIMs, i.e. abnormal, purposeless movements of the forelimb and digits contralateral to the lesion; orolingual AIMs, i.e. empty jaw movements and contralateral tongue protrusion; and locomotive AIMs, i.e. increased locomotion with contralateral side bias. Each of these four subtypes was scored on a severity scale from 0 to 4 (1, present during less than half of the observation time; 2, present during more than half of the observation time; 3, present all the time but suppressible by threatening stimuli; 4, present all the time and not suppressible). As reported previously (Lee *et al*., 2000), different AIM subtypes are preferably associated with different postures, because locomotion requires full contact of a rats' paws with the floor, and is not expressed when a rat stands on its hindlimbs. On the other hand, intense locomotor activity can mask adventitious movements of the limbs and paws. Thus, in order to allow for the scoring of locomotive AIMs in subjects that did not spontaneously express locomotion, animals were gently lifted up by their tail and forced to contact the floor of the cage with the forepaws. Conversely, expression of purposeless, abnormal limb movements in a locomotor-activated rat was searched for by gently lifting up its upper body, thus freeing the forelimbs from being engaged in supporting floor contacts (Lee *et al*., 2000). The theoretical maximum score that could be accumulated by one animal in one testing session was 144 (maximum score per observation point, 16; number of observation points per session, 9).

Cylinder test

Forelimb akinesia was assessed using a modified version (Kirik *et al*., 2000) of a test originally described by Schallert & Tillerson (2000). This test assesses a rat's independent forelimb use to support the body against the walls of a cylindrical enclosure. The test takes advantage of the animals' innate drive to explore a novel environment by standing on the hindlimbs and leaning towards the enclosing walls. To perform this test, rats were put individually in a glass cylinder (21 cm diameter, 34 cm height) and videorecorded for 5–7 min. No habituation to the cylinder prior to filming was allowed. The test was performed from between 10.00 h and 16.00 h. To stimulate rats that showed little or no tendency to explore, the following methods were used in this given order: (i) turning the lights in the room on and off 2–3 times, and then leaving them off while only a red light bulb was used as a source of illumination; (ii) mildly shaking the cylinder for 2–3 s (red light on); (iii) taking the rat out of the cylinder for ≈ 30 s and then putting it back. These manouevres were found to be more effective than other types of activating stimuli (e.g. exposing the rats to stimulating odours or mild stressors), which were preliminarily tested in a group of normal rats. Videorecordings were analysed by an investigator who was unaware of the rats' group membership and pharmacological treatment. The number of wall contacts performed independently with the left and the right forepaw were counted and noted down up to a total number of 20 wall contacts per rat and session. Only supporting contacts were counted, i.e. full appositions of the paws with open digits to the cylinder walls.

Rotometry

Rotational behaviour was measured by placing rats in hemispherical perspex bowls where they were tethered to an automated rotometer. Each 180° turn performed by an animal was fed to a PC computer running a photobeam activity system. Animals were allowed to habituate to the rotometer bowls for 5–10 min prior to recording. Rotation tests lasted for 3 or 5 h, as specified below (see Results), during which the number of left and right turns was recorded by the computer in 5- or 9-min bins, respectively. Testing was performed from between 10.00 h and 16.00 h.

Rotation diameter

The diameter of rotation was measured on a single testing session from rats that had been selected for the acute drug challenge study. A circular arena was constructed by apposing a circular length of metal (15 cm high, 50 cm in diameter) on a cardboard plate. On the cardboard, we drew eight concentric circles, 5 cm apart, starting at 5 cm from the enclosing metal wall. Rats were marked with a black ink dot on the middle of their back, allowed to habituate for 2 min within the arena, and then filmed for 8 min using a videocamera that was placed at a distance of ≈ 130 cm above the rat. Videorecordings were analysed in order to determine the diameter of the first 20 consecutive turns performed by an animal. The start of a turn was set

at the position where a rat's snout pointed at 12 o'clock on the monitoring screen; rotation diameter was calculated by measuring the distance between this and the farthest position reached by a rat upon completing one turn (the black ink dot was used as a reference; Fig. 6). Turns caused by side-falling were not counted. Injections of bromocriptine, L-DOPA and saline were made 120, 45 and 20 min, respectively, before the recordings started.

Expression of the data and statistical analysis

The relative responses to L-DOPA, bromocriptine and vehicle were identical in rats with instrastriatal or MFB lesions. These two lesion types were therefore pooled together within each of the three treatment arms under investigation (i.e. L-DOPA, bromocriptine and vehicle). Further details on the two lesion models are provided elsewhere (C. Winkler, D. Kirk, A. Björklund and M. A. Cenci, unpublished observations).

In the preliminary pharmacodynamic study, comparisons between the motor effects of L-DOPA, different doses of bromocriptine and vehicle were carried out using one-factor analysis of variance (ANOVA) followed by *post hoc* Tukey test. In the chronic drug treatment study, comparisons of motor parameters that had been recorded repeatedly (AIM scores; contralateral turns, peak rotational rate, rotational asymmetry; limb use asymmetry and no. of wall contacts in the cylinder test) were performed using repeated-measures ANOVA, where treatment (L-DOPA, bromocriptine or vehicle) and time (testing session) were entered as independent variables. *Post hoc* comparisons were performed where appropriate using the Tukey test. When examining acute challenges with antidyskinetic compounds, the motor performance measured after injecting a given compound was expressed as a percentage of that recorded from the same animal after administration of vehicle on the preceding or subsequent testing day. Statistical comparisons were performed using Student's paired *t*-test. Statistical significance level was set at $P < 0.05$. Data are expressed as means ± one SEM.

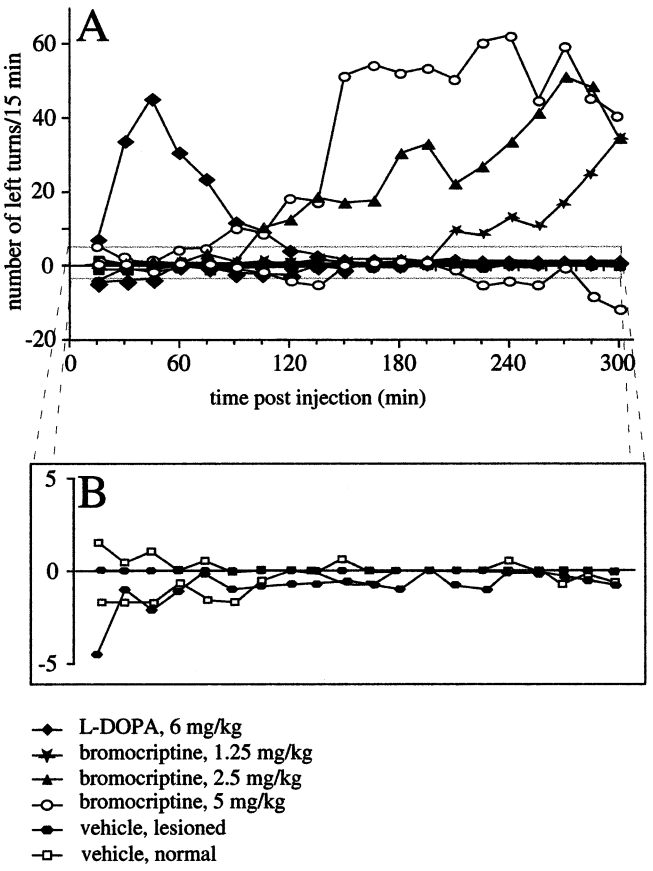

FIG. 1. (A) Pharmacodynamic profiles of L-DOPA, different doses of bromocriptine, and vehicle in a 5-h rotation test. (B) The performance of vehicle-injected normal and 6-OHDA-lesioned animals is shown at higher magnification. Turns in the direction contralateral to the lesion (left) are indicated by positive figures on the *y* axis.

Results

L-DOPA and bromocriptine have different pharmacodynamic profiles

Before undertaking a large-scale study, we set out to compare the motor effects of different doses of bromocriptine and L-DOPA. Drug-naïve rats were tested for 5 h in an automated rotometer after the acute injection of either 6 mg/kg L-DOPA ($n = 6$ rats with MFB lesions), 1.25, 2.5 or 5 mg/kg bromocriptine ($n = 5$–6 MFB-lesioned rats per dose), or the vehicle used to dissolve bromocriptine ($n = 16$, i.e. eight MFB lesions plus eight normal controls).

As shown in Fig. 1, the temporal course of motor activation was found to differ markedly among the groups. When challenged with vehicle, both normal and 6-OHDA-lesioned rats showed a small rotational response, which was maximal during the first hour postinjection (Fig. 1B). L-DOPA induced a monophasic rotational peak at 45 min, gradually returning to baseline by ≈140 min postinjection. Bromocriptine had a slow onset of action, as no motor activation was ever detected during the first 45 min postinjection (Fig. 1). The motor response to 5 and 2.5 mg/kg bromocriptine gradually increased during the first 2–3 h postinjection, and persisted thereafter at maximal levels until the end of the testing session. The effects produced by 1.25 mg/kg bromocriptine had a longer latency and slower temporal progression (Fig. 1). These pharmacodynamic differences between the drugs under investigation were taken into account in the choice of appropriate postinjection intervals for the different behavioural recordings (Table 1).

Regarding the motor stimulant effect of different drug doses, 2.5 mg/kg bromocriptine was found to produce the same amount of rotation as 6 mg/kg L-DOPA, whereas 1.25 and 5 mg/kg bromocriptine were found to have a weaker and stronger effect, respectively (Table 2). A cylinder-test session performed on the same animals (not shown) also indicated that doses of bromocriptine ranging between 2.5 and 3.5 mg/kg were suitable to be compared with 6 mg/kg L-DOPA in the body of the study.

Axial, limb and orolingual AIMs were induced by L-DOPA but not bromocriptine

During the chronic drug treatment period, L-DOPA but not bromocriptine induced a gradual development of AIMs affecting the trunk, limb and orolingual region (Fig. 2A; $P = 0.003$ for treatment effect; $P = 0.0003$ for time effect; $P < 0.0001$ for time and treatment interaction). In each testing session, axial, limb and orolingual AIM scores were at least 10-fold larger in L-DOPA- than bromocriptine-injected animals ($P < 0.05$; *post hoc* Tukey test). Within the L-DOPA group, body AIM scores showed a steady increase from the first to the last testing session (Fig. 2A; $P < 0.01$ for within-group comparisons between session 1 and 4, 5 or 6; *post hoc* Tukey test). Mild limb and axial AIMs (severity grade 2) were recorded in five out of 14 bromocriptine-treated rats. However,

TABLE 2. Rotational effects of different doses of bromocriptine as compared with 6 mglkg L-DOPA or vehicle

	Normal rats, vehicle	MFB lesions, vehicle	MFB lesions, L-DOPA + benserazide (6 + 15 mg/kg)	MFB lesions, 1.25 mg/kg bromocriptine	MFB lesions, 2.50 mg/kg bromocriptine	MFB lesions, 5.00 mg/kg bromocriptine
Rotational asymmetry (contralateral turns as percentage of total)	35.19 ± 5.81	187 ± 0.93	78.00 ± 19.89*	31.91 ± 15.06†	73.94 ± 12.76*	87.59 ± 11.32*‡
Peak rotational rate (right + left turns/min)	0.34 ± 0.06	0.85 ± 0.19	4.28 ± 1.87	2.37 ± 2.21	5.65 ± 1.90	7.35 ± 2.63‡

In a preliminary experiment, drug-naive animals were used to compare the motor effects of different doses of bromocriptine and L-DOPA. The results reported here were collected during a 5-h rotation test (see Fig. 1). Peak rotational rate was calculated at the 15-min recording bin during which each individual animal had shown the highest total number of turns. Data represent means ± SEM from 5–8 rats per group. $P < 0.05$ vs. *MFB lesion-vehicle; ‡normal-vehicle; †5.00 mg/kg bromocriptine (one-factor ANOVA and *post hoc* Tukey test.)

bromocriptine-treated animals did not differ significantly from vehicle-injected rats, which scored 0 on all AIM subtypes throughout the experiment (Fig. 2A and B). Neither orofacial nor limb AIMs of notable amplitude and severity were ever detected in bromocriptine-treated rats, not even when the animals were challenged with lower (1.25) or higher (5 mg/kg) drug doses after completion of the chronic drug treatment experiment (data not shown).

In contrast to body AIMs, locomotive AIM scores were significantly larger after treatment with bromocriptine compared with L-DOPA (Fig. 2B; $P = 0.044$ for treatment effect; $P < 0.05$ for bromocriptine vs. both L-DOPA and vehicle on testing sessions 2, 3 and 4). It is, however, worth noting that the severity grade of locomotive AIMs on single monitoring periods did not differ between bromocriptine- and L-DOPA-treated animals (data not shown). Thus, the large scores per session recorded in the bromocriptine group can be attributed to the sustained action of the drug during the postinjection interval chosen for testing (cf Fig. 1 and Table 1).

Physiological motor performance declined during the course of L-DOPA but not bromocriptine treatment

In this study, the cylinder test was used to provide a measure of normal, spontaneous motor behaviour to be compared with both AIMs and rotation (see Discussion). When all the 6-OHDA-lesioned rats were tested off drugs prior to the onset of chronic drug treatment ('baseline' in Fig. 3), the proportion of wall contacts performed by the parkinsonian (left) paw amounted to ≈ 15–25% of total (Fig. 3A). Note that animals allotted to the bromocriptine-, L-DOPA- and vehicle groups did not differ significantly from each other ($P = 0.197$; one factor ANOVA). In the chronic drug treatment experiment, L-DOPA and bromocriptine produced a similar improvement in limb use asymmetry, raising the percentage of wall contacts performed by the parkinsonian paw to 40–45% of total (Fig. 3A; $P = 0.020$ for group effect, $P < 0.01$ for both L-DOPA and bromocriptine vs. vehicle in all testing sessions). The percentage of left-paw contacts remained overall stable across the treatment period (Fig. 3A; $P = 0.256$ for time effect; $P = 0.915$ for time and treatment interaction). However, changes occurred with respect to the general levels of performance in this test (Fig. 3B and C). The absolute number of wall contacts performed by L-DOPA-treated animals gradually declined from the first to the last testing session (see left paw contacts in Fig. 3B and total wall contacts in Fig. 3C; $P < 0.01$ for a within-group comparison between session 1 and 3 in both diagrams). Such a decline was closely associated with the development of body AIMs during the treatment period. Indeed, the number of wall contacts performed on the third cylinder-test session was

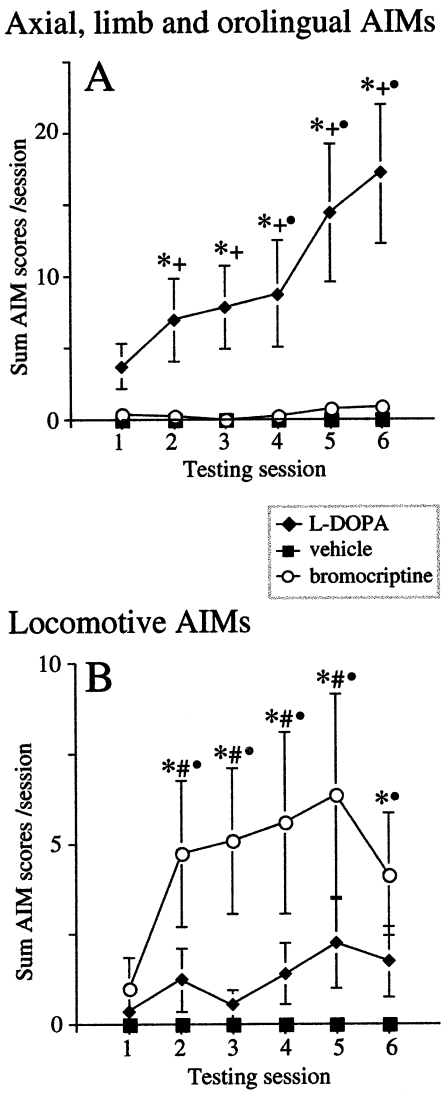

FIG. 2. Chronic treatment with L-DOPA but not bromocriptine induces abnormal involuntary movements (AIM) of the trunk, limb and orolingual region. AIM scores were recorded from unilaterally 6-OHDA-lesioned animals which received a 3-week treatment with L-DOPA (6 mg/kg/day combined with benserazide, 15 mg/kg/day; $n = 14$), bromocriptine (3.5 mg/kg/day; $n = 14$) or vehicle ($n = 8$). The animals were tested for AIMs twice a week. (A) The sum of axial, limb and orolingual scores recorded in each testing session; (B) locomotive AIM scores. $P < 0.05$ vs: *vehicle; +bromocriptine; #L-DOPA; •testing session 1 in the same group.

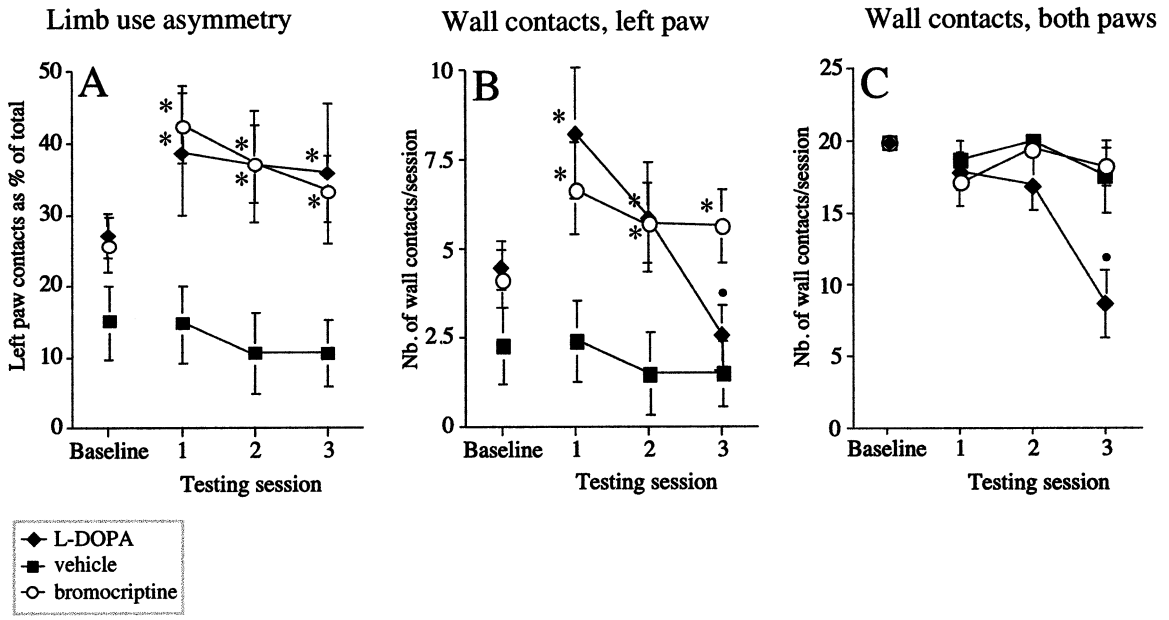

FIG. 3. Performance in the cylinder test during a 3-week course of treatment with L-DOPA (6 mg/kg/day combined with benserazide, 15 mg/kg; $n = 14$), bromocriptine (3.5 mg/kg/day; $n = 14$) or vehicle ($n = 8$). (A) Limb use asymmetry, i.e. the percentage of wall contacts performed with the parkinsonian (left) paw. (B,C) The absolute number of wall contacts performed with (B) this or (C) both paws. Animals affected by severe L-DOPA-induced AIMs (grade 4) performed 0 wall contacts. This caused the exclusion of some animals from the computation of limb use asymmetry (A) in testing sessions 2 (1 case) or 3 (5 cases). $P < 0.05$ vs: *vehicle; •testing session 1 in the same group.

inversely correlated with the animals' axial, limb and orolingual AIM scores (Fig. 4A). These data reflect a mutually exclusive relationship between AIMs and physiological motor performance. Indeed, the parkinsonian forelimb was never used to support the animal's body against the cylinder walls while it was being affected by dyskinetic movements. Moreover, severe body AIMs (grade 4) completely replaced an animal's normal behaviour, and could not be interrupted by any activating stimuli. Bromocriptine-injected animals, which developed increasingly severe rotation (see below) but not AIMs of the head, limb and trunk, maintained a stable performance in the cylinder test throughout the experiment (Fig. 3B and C). It is worth noting that the interference with and/or replacement of normal motor activity by AIMs is a key feature of L-DOPA-induced dyskinesia, and provides a basis for dyskinesia severity ratings in both nonhuman primate models (Pearce *et al.*, 1998) and parkinsonian patients (Hagell & Widner, 1999; references therein).

Contralateral rotation increased over time in both L-DOPA- and bromocriptine-treated animals

During the chronic drug treatment study, both L-DOPA and bromocriptine induced increasing levels of contralateral turning (Fig. 5A; $P = 0.013$ for treatment effect; $P = 0.013$ for time effect), but this effect was much stronger in bromocriptine-treated animals (Fig. 5A; $P = 0.041$ for time and treatment interaction; $P < 0.05$ for a comparison between bromocriptine and L-DOPA or vehicle in each session). Because these high rotational scores were likely to reflect, at least in part, the very sustained action of bromocriptine during the testing interval (cf. Fig. 1), we further compared the treatment groups on two rotational parameters that are not dependent on pharmacodynamic factors, i.e. peak rotational rate and rotational asymmetry. Peak rotational rate was defined as the maximal no. of turns per recording bin (9 min) displayed by each individual animal. Comparisons of turning rates confirmed that bromocriptine had an overall stronger stimulatory action on rotation than did L-DOPA or vehicle (Fig. 5B; $P = 0.005$ for treatment effect; $P < 0.05$ for bromocriptine vs. L-DOPA or vehicle in sessions 2–6). However, there was no difference between bromocriptine and L-DOPA upon their first, acute administration (see session 1 in Fig. 5B), which is in line with the results of our preliminary experiment (Table 2). A difference between the two treatment groups became apparent on testing session 2, and grew even larger later on, due to a gradual increase in the rates of bromocriptine-induced rotation (Fig. 5B; $P = 0.008$ for time effect; $P = 0.001$ for time and treatment interaction; $P < 0.01$ for a within-group comparison between the first and all the other testing sessions in the bromocriptine group).

Rotational asymmetry was defined as the percentage of contralateral turns in a session (Fig. 5C). As expected, both bromocriptine and L-DOPA reversed the lesion-induced ipsilateral turning bias in each session (Fig. 5C; $P = 0.010$ for treatment effect, $P < 0.05$ for both bromocriptine and L-DOPA vs. vehicle in sessions 1–6). In the animals injected with L-DOPA (but not bromocriptine) the proportion of contralateral turns increased gradually during the course of the treatment (Fig. 5C; $P = 0.020$ for time effect; $P = 0.002$ for time and treatment interaction; $P < 0.01$ for a within-group comparison between sessions 1 and 6). This increase was significantly correlated with the development of AIMs (Fig. 4B; note that there was, however, no significant correlation between AIMs and absolute numbers of contralateral turns; Fig. 4C).

Taken together, these data show that the rats' contralateral turning response, whether induced by L-DOPA or bromocriptine, shows sensitization (i.e. a gradual increase over time upon administration of the same drug dose), which is in contrast with the drug-induced improvement in physiological motor activities (see cylinder test above). Differently from previous studies in unilaterally 6-OHDA-

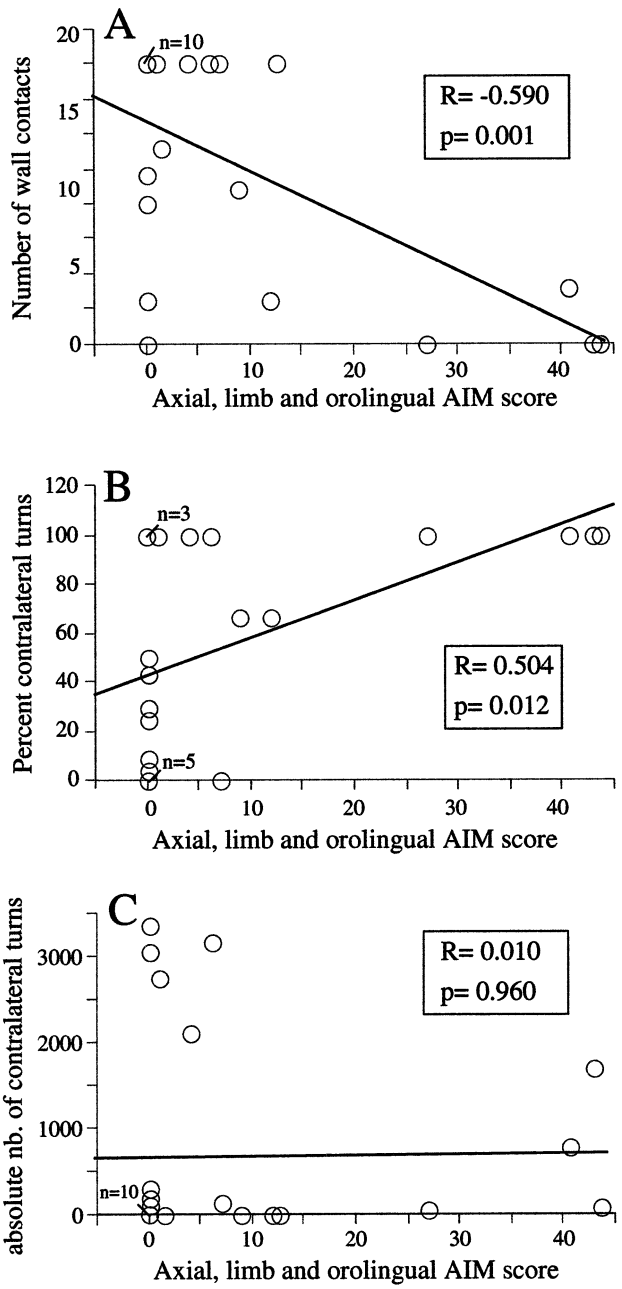

FIG. 4. (A) Axial, limb and orolingual AIM scores are inversely correlated with the animals' performance in the cylinder test, and (B) positively correlated with their degree of rotational asymmetry (percentage of contralateral turns). (C) There is however, no correlation between AIMs and absolute numbers of contralateral turns. The results shown here were obtained on three consecutive testing days during the third week of chronic drug treatment (day 1, AIM ratings; day 2, cylinder test; and day 3, rotational recordings). In A, B and C, the *x*-axis shows the sum of axial, limb and orolingual AIM scores recorded during one testing session. The *y*-axis shows (A) total number of wall contacts in the cylinder test, and (B) percentage or (C) absolute number of contralateral turns in the rotometer session. All drug-treated animals (bromocriptine and L-DOPA groups) were included in these computations (similar correlations were obtained by including also the vehicle-treated controls; not shown). R, Pearson's correlation coefficient.

lesioned rats (Henry *et al.*, 1998), these results also show that bromocriptine has the potential to produce much higher levels of contralateral rotation than does L-DOPA.

The phenomenology of rotation differed between bromocriptine and L-DOPA treatment

When animals were placed in a large circular arena, bromocriptine-injected rats featured free but asymmetrical locomotion, performing wide turns at a running pace. By contrast, L-DOPA-treated animals exhibited slower and tight turns, twisting around a vertical axis (cf Fig. 6A and B). Thus, turn diameter was significantly larger in bromocriptine- than L-DOPA-treated animals (Fig. 6C). These results show that the phenomenology of drug-induced rotation differs markedly between bromocriptine and L-DOPA, reflecting a difference in the underlying neural mechanisms. Tight but not wide turns are associated with a twisted, dystonic body posture. These data indicate that, unlike traditional rotometers, video-based systems for the analysis of rotation may be useful in studies that aim at clarifying the neural mechanisms of a rat's turning response. This type of analysis would also be useful for the screening of antiparkinsonian compounds with different dyskinesiogenic potential.

L-DOPA-induced AIMs but not rotation were attenuated by antidyskinetic compounds

After the chronic drug treatment experiment, 6–7 rats from each the L-DOPA, bromocriptine and vehicle groups were kept on a maintainance regimen consisting of 2–4 injections per week of the previously administered treatment. During this part of the study, we assessed the ability of five antidyskinetic compounds to affect drug-induced AIMs, rotation and performance in the cylinder test. An overview of these results is provided in Table 3. The following account will focus on the most consistently affected motor parameter, i.e. L-DOPA-induced AIMs. Differential effects of the tested drugs on the time–action curve of L-DOPA are shown in Fig. 7. The average reduction in AIM scores during the testing interval is reported in Table 3. As shown in Table 3, compounds (and/or doses of) that significantly attenuated L-DOPA-induced AIM scores did not produce a parallel effect on either the number of turns or the rotational rates recorded from the same animals.

Naloxone

The nonselective opioid receptor antagonist naloxone has been shown to alleviate L-DOPA-induced dyskinesia in parkinsonian patients (Trabucchi *et al.*, 1982; Sandyk & Snider, 1986). When given at 4 and 8 mg/kg, naloxone attenuated L-DOPA-induced axial, limb and orolingual AIMs by ≈ 19 and 17%, respectively ($P = 0.020$ for L-DOPA + naloxone vs. L-DOPA + vehicle; $n = 7$). L-DOPA-induced locomotive AIMs were, however, not reduced ($P = 0.143$; Table 3). The antidyskinetic effect of naloxone was mainly evident at the beginning and end of the L-DOPA time–action curve (Fig. 7A), whereas the peak of dyskinesia severity at 80–100 min post L-DOPA-injection was not affected.

Yohimbine

The α_2 adrenoreceptor antagonists, such as yohimbine and idazoxan, have been shown to reduce L-DOPA-induced dyskinesia in MPTP-treated monkeys (Gomez-Mancilla & Bédard, 1993; Henry *et al.*, 1999; Grondin *et al.*, 2000), and in parkinsonian patients (Rascol *et al.*, 2001; but see Manson *et al.*, 2000).

When tested at a dose of 10 mg/kg, yohimbine caused a dramatic attenuation of all AIM subtypes in L-DOPA-treated animals ($P = 0.025$; Table 3). The antidyskinetic effect of yohimbine was evident during all phases of the L-DOPA time–action curve (Fig. 7B). However, this dose of yohimbine had a deleterious effect of the animals' overall motor activity, and produced severe dyspnoea,

TABLE 3. Effects of nondoparninergic agents in combination with L-DOPA, bromocriptine or saline treatment

	L-DOPA treatment				Bromocriptine treatment				Saline treatment	
	ALO AIMs	Lo AIMs	Rotation	Limb use	ALO AIMs	Lo AIMs	Rotation	Limb use	Rotation	Limb use
Naloxone 4 mg/kg	–19 ± 4%	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Naloxone 8 mg/kg	–17 ± 6%	n.s.	n.a.	n.s.	n.s.	+30 ± 8%	n.a.	+8 ± 8%	n.a.	n.s.
Yohimbine 10 mg/kg	–91 ± 5%	–70 ± 17%	n.a.	n.s.	n.s.	n.s.	n.a.	n.s.	n.a.	n.s.
Yohimbine 1 mg/kg	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	–20 ± 4%	n.s.	n.s.	n.s.
MDOT 2 mg/kg	–37 ± 7%	–47 ± 6%	n.s.	n.s.	n.s.	n.s.	–49 ± 11%	n.s.	n.s.	n.s.
Clozapine 8 mg/kg	30 ± 9%	n.s.	n.s.	n.a.	n.s.	n.s.	n.s	n.a.	n.s.	n.a.
Amantadine 40 mg/kg	50 ± 10%	n.s.	n.s.	n.a.	–83 ± 17%	–100 ± 0%	–58 ± 32%	n.a.	n.s.	n.a.

Overview of the effects produced by antidyskinetic compounds. Values represent percentage increases (+) or decreases (–) relative to the performance recorded after injection of the same vehicle in which the challenging drug had been dissolved. Only changes that reached statistical significance (paired *t*-test) are reported (n = 6–7 per group). n.s., nonsignificant difference; n.a., not applicable (the test was not performed). ALO AIMs, axial, limb and orolingual abnormal involuntary movements scores; Lo AIMs, locomotive abnormal involuntary movements scores; Limb use, limb use asymmetry in the cylinder test (i.e. left paw contacts as percentage of total); Rotation, number of contralateral turns recorded by automated rotometry (similar results were obtained by an analysis of peak rotational rates).

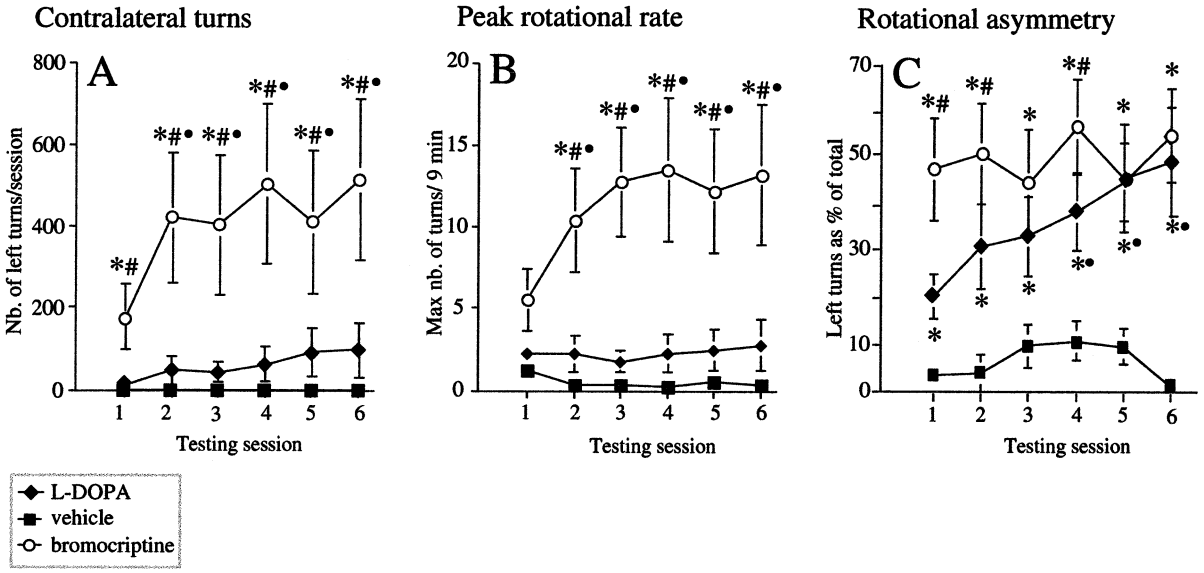

FIG. 5. Rotometry measurements during a 3-week course of treatment with L-DOPA (6 mg/kg/day combined with benserazide, 15 mg/kg; n = 14), bromocriptine (3.5 mg/kg/day; n = 14) or vehicle (n = 8). (A) Total numbers of turns in the direction contralateral to the lesion; (B) peak rotational rates; (C) rotational asymmetries (i.e. percentages of contralateral turns in each recording session). $P < 0.05$ vs: *vehicle; #L-DOPA; •testing session 1 in the same group.

causing the death of one animal within the L-DOPA group. For this reason, 10 mg/kg yohimbine was not tested further, and we turned to testing 1 mg/kg of this compound, which was devoid of adverse side-effects. This lower dose of yohimbine reduced L-DOPA-induced axial, limb and orolingual AIM scores by ≈30% in five out of the six tested animals, but its overall effect did not reach statistical significance (P = 0.059).

5-Methoxy 5-N,N-dimethyl-tryptamine (5-MDOT)

The 5-HT uptake inhibitor 5-methoxy 5-N,N-dimethyl-tryptamine (5-MDOT) has been shown to alleviate L-DOPA-induced dyskinesia in MPTP-treated monkeys (Gomez-Mancilla & Bédard, 1993). We tested one single dose of this compound (2 mg/kg), which has been reported to reduce L-DOPA-induced rotation in unilaterally 6-OHDA-lesioned rats (as assessed in 15-min-long rotometer sessions; Henry *et al*., 1998).

5-MDOT produced an ≈40–50% reduction in all AIM-score subtypes in the L-DOPA-treated group (P = 0.030; Table 3). 5-MDOT exerted its antidyskinetic action mainly by delaying the onset and reducing the duration of the L-DOPA effect, without affecting the peak severity of L-DOPA-induced AIMs (Fig. 7C).

(iv) Clozapine

The atypical neuroleptic, clozapine, which has antagonistic activity at mainly D1- and D4-type DA receptors (see, e.g. Murray & Waddington, 1990; Seeman, 1992), has been shown to alleviate L-

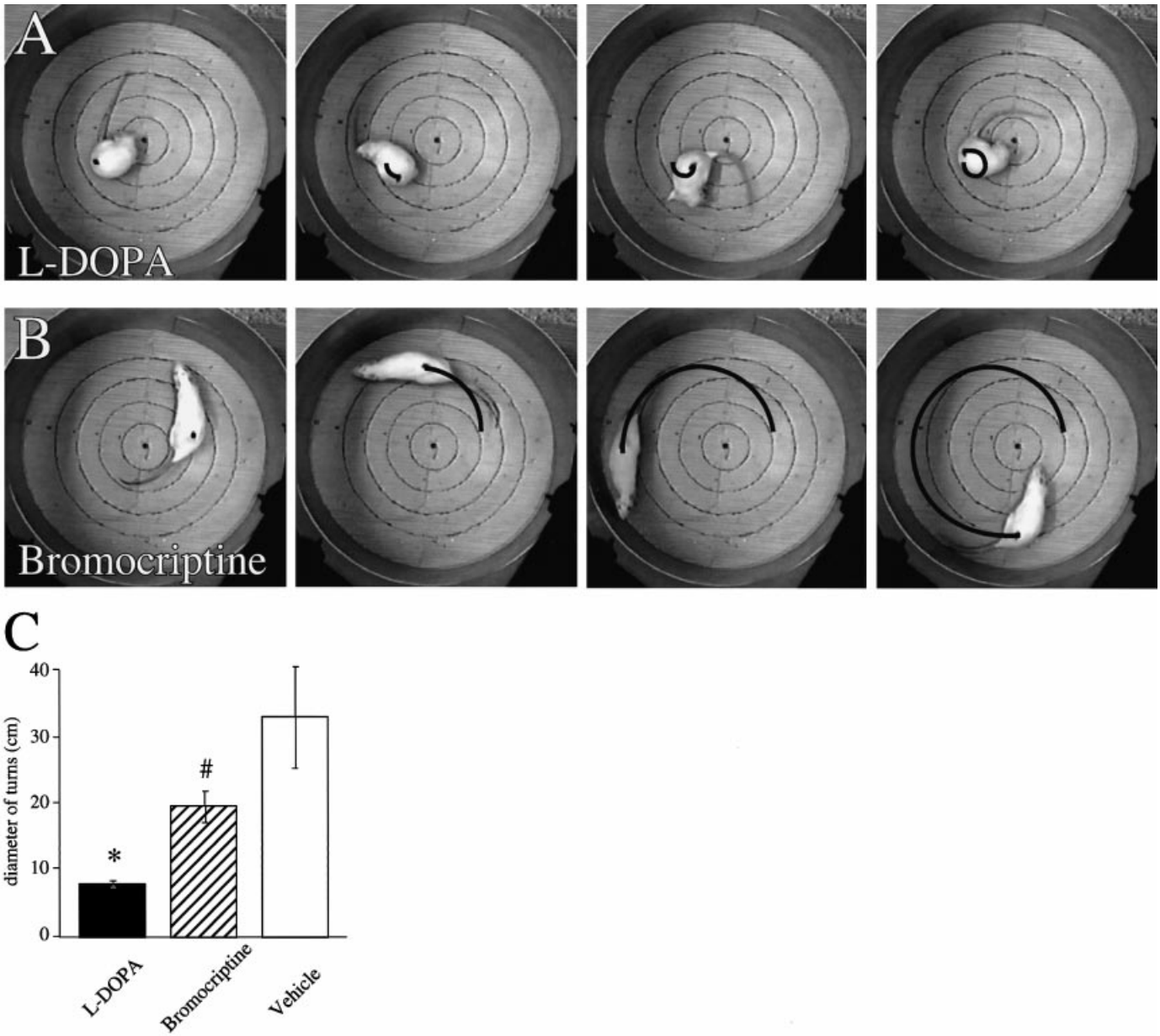

FIG. 6. Unlike traditional rotometry, video-based analysis of rotation can uncover the phenomenology of drug-induced turning. (A) Because of an abnormally twisted body posture, L-DOPA-treated animals perform very narrow turns. (B) In contrast, bromocriptine-treated animals show free, asymmetrical locomotion. (C) Accordingly, the diameter of the turns is significantly larger in animals treated with bromocriptine compared to L-DOPA. In A and B, a black line has been drawn along the track of the turn. The drug-treated groups in this dataset only comprise animals which had shown levels of rotation significantly above vehicle-injected rats (n = 5–8 per group). $P < 0.05$ vs: *vehicle; $^{\#}$L-DOPA.

DOPA-induced dyskinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys (Grondin *et al*., 1999) as well as parkinsonian patients (Bennett *et al*., 1993; Durif *et al*., 1997).

Clozapine was tested at doses that have been reported to reduce hyperkinetic motor manifestations without affecting physiological motor performance in rats (i.e. 4 and 8 mg/kg; see Chesler & Salamone, 1996; Trevitt *et al*., 1997). The lower dose of clozapine tested did not have any significant effect on drug-induced AIMs (data not shown). However, the higher dose produced an average 30% attenuation of axial, limb and orolingual AIMs in the L-DOPA group (P = 0.029; n = 6). L-DOPA-induced locomotive AIM scores were not affected (P = 0.620). The antidyskinetic effect of clozapine was mainly evident at the beginning and peak of the L-DOPA time–action curve (Fig. 7D).

(v) Amantadine

The antiglutamatergic agent amantadine has been shown to alleviate L-DOPA-induced dyskinesia in parkinsonian monkeys (Blanchet *et al*., 1998), and has a well-documented antidyskinetic effect in Parkinson's disease (see, e.g., Luginger *et al*., 2000; Snow *et al*., 2000; references therein).

Amantadine was tested a the single dose of 40 mg/kg, after assessment that such a dose did not produce gross alterations in motor performance when given alone (data not shown; see also Menon *et al*.,

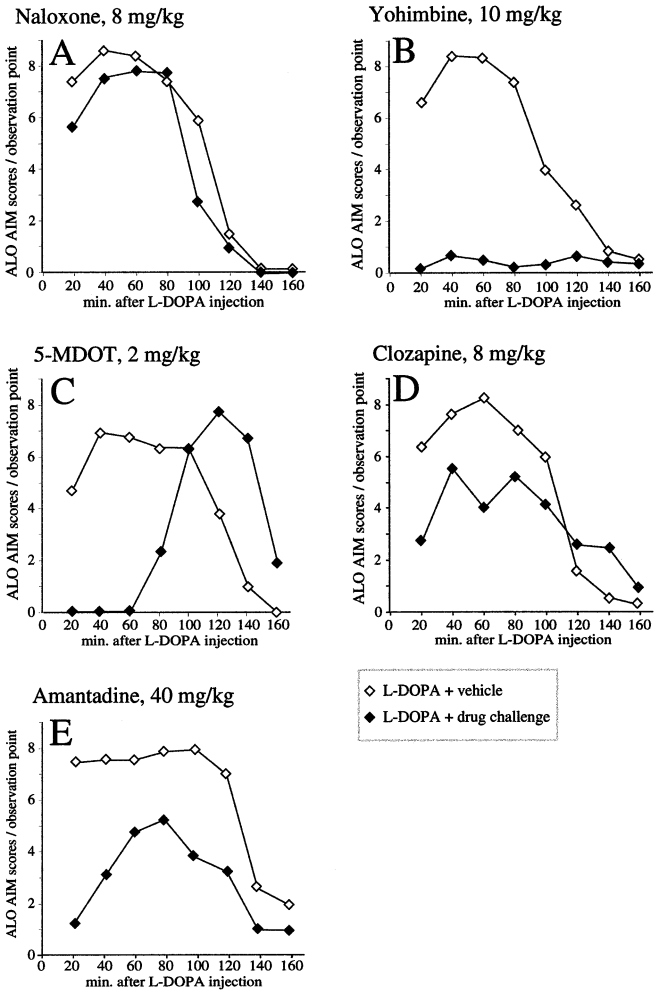

FIG. 7. Differential effects of five nondopaminergic compounds on the time–action curve of L-DOPA-induced AIMs: (A) naloxone; (B) yohimbine; (C) 5-MDOT; (D) clozapine; and (E) amantadine. The diagrams show the rise and fall of axial, limb and orolingual (ALO) AIMs scores after a single injection of L-DOPA, in combination with the challenging compound (◆) or its corresponding vehicle (◇). The temporal profile of rat AIMs simulates the time course of peak-dose dyskinesia in Parkinson's disease. Values give the average AIM score/monitoring period in all the tested animals (n = 6–7).

1973). The compound caused an ≈50% reduction in axial, limb and orolingual AIMs (P = 0.045; Fig. 7E), but not locomotive AIMs (P = 0.277) in the L-DOPA-treated animals.

Discussion

Rats with 6-OHDA lesions remain the most cost-effective animal model of Parkinson's disease for the screening of new treatments. Yet, there is currently little agreement on which behavioural tests in rats would provide measures that are predictive of a clinical benefit in patients. In the absence of commonly accepted guidelines, the choice of behavioural testing paradigms relies on the individual preferences of each investigator. However, not all behavioural measures are equally appropriate for all questions, and the choice of nonspecific and/or poorly characterized testing routines may eventually cast doubts on the clinical relevance of rat studies (Nutt, 1990; Bézard *et al*., 2001). Most investigators agree that animal models of neurological symptoms are valid to the extent that they reproduce key functional features of the corresponding human condition. The most conclusive demonstration of functional similarity would be obtained by testing the response of the model to the effects of interventions which are known to alleviate or exacerbate the corresponding human symptom (Schallert & Tillerson, 2000).

One of the most significant causes of disability in Parkinson's disease consists of a diminution and slowness of movement, associated with delayed movement initiation (akinesia). L-DOPA pharmacotherapy is very effective in alleviating these symptoms, but with time induces abnormal involuntary movements (dyskinesia) in the majority of patients (for review see Nutt, 1990). Dyskinesia can itself become a cause of disability because it disrupts the execution of purposeful motor acts. By analogy with the clinical symptoms and the corresponding models in nonhuman primates (see, e.g., Pearce *et al*., 1998; Grondin *et al*., 1999), akinesia in rats would be expected to manifest itself as a diminished expression of normal motor activities. Adequate tests of parkinsonian akinesia should be sensitive to the disrupting effects of DA-denervating lesions, and to the beneficial effects of antiparkinsonian drugs. Conversely, a valid test of drug-induced dyskinesia should be able to rate movements that are unambiguously abnormal and can disrupt physiological motor performance. Ideally, such a test should be sensitive to the effects of drugs or treatments which have a proven antidyskinetic action in parkinsonian patients, and/or in nonhuman primate models of dyskinesia. A chronic course of L-DOPA treatment would be expected to cause a progressive augmentation (sensitization) of dyskinesias, associated with an impairment in the animals' ability to attend to purposeful, adaptive motor behaviours.

The present study provides a conclusive demonstration that rats do exhibit both akinetic and dyskinetic motor deficits which fulfil the above functional requirements. In the first part of the study, we compared the motor effects of L-DOPA and bromocriptine, two antiparkinsonian drugs which cause a high vs. low incidence of dyskinesia when given to parkinsonian primates de novo (Rascol *et al*., 1979; Lees & Stern, 1981; Bédard *et al*., 1986; Pearce *et al*., 1998). We found that both drugs improved the rats' ability to use their parkinsonian forelimb in a test of spontaneous motor function (cylinder test). The antiakinetic action of the two drugs remained overall stable during the chronic treatment period. Rats treated with L-DOPA, but not bromocriptine, showed, however, a gradual decline in their absolute levels of performance in this test. Such a decline was caused by the development of abnormal movements affecting limb, trunk and orofacial muscles. These movements interfered with, and gradually replaced, the rats' normal motor activities. In contrast to cylinder-test performance, L-DOPA-induced AIMs showed sensitization, i.e. an increasing severity upon repeated administration of the same drug dose. Animals treated with bromocriptine did not express abnormal movements of the limb, trunk and orofacial region significantly above the levels measured in vehicle-injected controls. However, they showed higher levels of contralateral rotation than did L-DOPA-treated rats. During the second part of the study, we tested the effects of five compounds which have been shown to alleviate L-DOPA-induced dyskinesia in parkinsonian primates. All these compounds caused a significant reduction in the axial, limb and orolingual AIM scores, but not in the number of rotations recorded from L-DOPA-treated animals.

The present results warrant a comparison between the motor tests used in this study with respect to their significance and range of applicability.

The cylinder test was chosen here to assess physiological motor function because it offers several advantages. On a conceptual level,

the nature of the motor manifestation being tested is unequivocal: the movements observed are identical to those typically performed by a rat in its home cage, and are examined without experimenter handling (Schallert & Tillerson, 2000). The test is very simple, objective, fast in its execution and does not require animal pretraining or aversive motivation. As previously documented (Kirik *et al*., 2000; Schallert *et al*., 2000), this test is sensitive to the disrupting effects of DA-denervating lesions. We now provide demonstration that it is also sensitive to the motor improvement produced by antiparkinsonian compounds. Moreover, we show that drug-induced AIMs disrupt the animals' performance in this test. Because of all these positive features, the cylinder test appears suitable for a preclinical screening of candidate antiparkinsonian compounds in the rat. We should, however, also report a potential drawback of this test, which we have experienced in the present study. As a rat's exploratory drive diminishes upon repeated exposure to the testing environment, the absolute number of supporting wall contacts performed in each session will gradually decrease, resulting in a reduced sensitivity of the test over long-term studies. Despite a relatively low testing frequency (never more than two cylinder tests per week), we experienced this problem towards the end of the experiment, and were unable to restore good levels of performance, even though we tried testing the animals during their active night hours.

Abnormal involuntary movement (AIM) rating was carried out using a method that we have recently introduced (Cenci *et al*., 1998; Andersson *et al*., 1999; Lee *et al*., 2000; Johansson *et al*., 2001). We have previously reported that, in addition to the extensively described rotation, 6-OHDA-lesioned rats treated with L-DOPA develop abnormal and purposeless movements affecting several body regions. These movements can be quantified according to principles similar to those applied in clinical dyskinesia rating scales (Hagell & Widner, 1999; references therein). Our rating method assigns separate scores to three distinct topographical subtypes of abnormal movements (axial, limb and orolingual AIMs), and to circular locomotion (locomotive AIMs), using a severity scale that is based on the proportion of time during which the observed item is present. This rating method differs radically from ordinal dyskinesia or stereotypy scales previously used in rats, where numbers denoted the presence or absence of different response categories, such as chewing, licking, and grooming (Creese & Iversen, 1973; Lindner *et al*., 1996). Moreover, only movements that are clearly abnormal for the rat are classified as dyskinetic, whereas enhanced manifestations of normal motor activities (grooming, gnawing, rearing and sniffing) are not included in the rating. Rat AIMs share similar cellular and molecular correlates to L-DOPA-induced dyskinesia in primates, e.g. an association with increased striatal levels of opioid precursor mRNAs (Brotchie *et al*., 1998; Cenci *et al*., 1998) and FosB/ΔFosB-related proteins (Doucet *et al*., 1996; Andersson *et al*., 1999) and with altered levels of opioid receptor binding in cortical and basal ganglia regions (Johansson *et al*., 2001; Piccini *et al*., 1997). This study provides the first demonstration that rat AIMs also share similar pharmacological properties to primate models of L-DOPA-induced dyskinesia, i.e. (i) induction by L-DOPA but not de novo bromocriptine; (ii) attenuation by several nondopaminergic compounds with proven antidyskinetic efficacy in patients and/or nonhuman primates. The compounds tested here affected different neurotransmitter receptors and systems (noradrenaline, serotonine, dopamine, glutamate, and opioids). Although the mechanisms underlying the antidyskinetic action of these drugs are poorly understood (for review see Rascol *et al*., 1999), the present data prove that there is a fundamental similarity between rats and primates with respect to the neurotransmitter systems controlling the expression of dyskinesia. Differently from axial, limb and orolingual AIMs, locomotive AIMs were induced by both bromocriptine and L-DOPA and, similar to rotation, showed little sensitivity to the antidyskinetic drugs tested (see Table 3). The present data underscore a close relationship between what we define as 'locomotive AIMs' and classic rotation. It should, however, be pointed out that ratings of circular locomotion based on a direct observation of the animals are not equivalent to automated rotometry. Our locomotive AIM rating only takes into account movements whereby a rat moves in circles using all four limbs. A rotometer would certainly provide a measure of this type of movement, but it would also count turns that lack a locomotor component, e.g. those produced by side falling. There are other important qualitative differences between rotometry measurements and locomotive AIMs scores. Our rating scale defines the severity of circular locomotion based on its frequency, persistence and degree of suppressability. On the other hand, rotometry measurements are mostly sensitive to the velocity of turning. These methodological aspects can explain why, in this study, drugs that reduced locomotive AIMs scores did not always reduce rotation, and vice versa (see, e.g. effects of yohimbine and 5-MDOT in Table 3).

The phenomenon of rotation was first described by Ungerstedt in the early seventies (Ungerstedt & Arbuthnott, 1970; Ungerstedt, 1976). Animals with unilateral 6-OHDA lesions of the MFB rotate ipsilaterally following administration of compounds that release DA, and contralaterally following treatment with L-DOPA or DA-receptor agonists. Ungerstedt interpreted these findings as indicating that a rat turns away from the side where DA activity is greater (Ungerstedt, 1976). The mechanisms by which asymmetry in central DA systems translates into the motor response of turning remain, however, unclear (for review see Miller & Beninger, 1991; Dunnett & Robbins, 1992). Moreover, there is currently no consensus on the behavioural components underlying turning, which has been interpreted either as a lateralized expression of sensory hyperattention or as the result of asymmetries in the dopaminergic control of posture or locomotion (for review see Schwarting & Huston, 1996; Miller & Beninger, 1991).

The results of the present study further highlight the nonspecificity of rotation as a measure of behavioural outcome in the rat Parkinson model. A comparison between rotometry and performance in the cylinder test reveals that drug-induced turning does not parallel an improvement in physiological motor function. Whilst forelimb akinesia was ameliorated (albeit not restored to normal levels) by both bromocriptine and L-DOPA, rotation was stimulated by the same drugs to an abnormal extent, which greatly exceeded physiological turning responses in vehicle-injected rats (reported in Fig. 1 and Table 2). Moreover, contralateral rotation showed a progressive increase (sensitization) during a course of drug treatment, while the rats' ability to perform purposeful motor acts either remained stable (bromocriptine) or declined (L-DOPA). These data show that rotation is not suitable for monitoring antiakinetic effects of antiparkinsonian compounds in the rat. For this purpose, it is preferable to measure improvements in adaptive motor behaviours that are clearly associated with the relief of parkinsonian motor features. On the other hand, rotation cannot be regarded as a valid measure of dyskinesia either, because (i) it is induced to a very large extent by drugs that have very low dyskinesiogenic potential, such as bromocriptine, and (ii) it is not attenuated by compounds that have antidyskinetic efficacy in patients. Our data are in contrast with a recent study in 6-OHDA-lesioned rats, showing a much more prominent induction of contraversive turning by L-DOPA compared with bromocriptine, and an attenuation of L-DOPA-induced turning by several antidyskinetic drugs, including some of the compounds tested here (Henry *et al*., 1998). The

discrepancy between these and our data can easily be explained by the different sampling intervals used for rotational recordings. In Henry *et al*. (1998), bromocriptine-induced rotation was measured at 0–120 min postinjection, i.e. before the drug reached its full effect, whilst antidyskinetic compounds were tested in very short recording sessions (5–20 min post drug-injection) which did not cover the time–action curve of L-DOPA.

In summary, drug-induced rotation is a hyperkinetic response of uncertain conceptual value, which may reflect the supersensitivity of brain DA receptors after DA denervation (for review see Miller & Beninger, 1991), but does not reproduce the pharmacology of L-DOPA-induced dyskinesia in primates. Rotational tests offer a number of practical advantages as they are fast, simple, objective and repeatable over longitudial studies. They provide an excellent and established method of monitoring the effects of treatments which deplete or enhance DA fibre afferents to the striatum, such as lesions and transplants. However, the clinical relevance of simple rotational recordings is limited by their inability to dissociate between therapeutically useful and dyskinetic effects of antiparkinsonian treatments.

Novel treatments for Parkinson's disease will be successful to the exent that they can relieve akinesia without inducing abnormal excessive movement. As previously pointed out (Schallert, 1995; Lindner *et al*., 1996), meaningful preclinical screening of these treatments in rats is conditioned by the selection of appropriate behavioural tests. This study demonstrates that clinically relevant measures of parkinsonian akinesia or dyskinesia can indeed be obtained in rats, and provides an example of how pharmacological tools can be used to interpret motor manifestations in parkinsonian rodents.

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Abbreviations

5-MDOT, 5-methoxy 5-N,N-dimethyl-tryptamine; 6-OHDA, 6-hydroxydopamine; AIM, abnormal involuntary movement; CPu, caudate–putamen; DA, dopamine; MFB, medial forebrain bundle; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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