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Further validation of LABORAS™ using various dopaminergic manipulations in mice including MPTP-induced nigro-striatal degeneration

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Abstract

The automated behavioural apparatus, LABORAS™ (Laboratory Animal Behaviour Observation, Registration and Analysis System), has been further validated with respect to the ability of the system to detect behavioural impairments in mice, following various dopaminergic manipulations. Initially data were obtained from mice administered with amphetamine, haloperidol, SCH23390, apomorphine and L-DOPA, with the focus on locomotor and grooming activities. The data recorded by LABORAS™ on administration of these pharmacological tool compounds, is comparable with published findings using standard LMA systems and conventional observer methods. In addition the home cage behaviour of mice administered with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) using an acute dosing regimen was also investigated. In LABORAS™, mice subjected to MPTP lesioning showed deficits in spontaneous motor activity at day 6–7 post-MPTP administration, over a 24 h test period, as compared to saline treated controls. The data captured and analysed using LABORAS™, suggests that the automated system is able to detect both pharmacologically and lesion-induced changes in behaviour of mice, reliably and efficiently.

Keywords: Mice; Automatic behaviour registration; Dopamine agonists; Dopamine antagonists; L-DOPA; MPTP

1. Introduction

LABORAS™ (Laboratory Animal Behaviour Observation, Registration and Analysis System), is an automated behavioural classification system, that has been developed to register the duration and frequency of feeding, drinking, rearing, climbing, immobility, locomotor activity (LMA) and grooming behaviours in mice and rats. This home–cage system can monitor rodent behaviour over a 24 h activity period for up to 7 days. Unique from other behavioural registration systems, LABORAS™ is based on transposing the mechanical vibrations caused by the movement of an individually housed experimental animal into electrical signals, which are scored and distinguished by a computer into the various behavioural categories, in accordance with unique-amplitude and frequency patterns (Schlingmann et al., 1998).

Previously, baseline behavioural data from a variety of pharmacologically naïve rodent strains has been recorded using LABORAS™ and compared with data obtained from conventional observer methods (Van de Weerd et al., 2001). In addition, further validation studies have been carried out to assess the ability of LABORAS™ to detect both pharmacological and lesion-induced behaviours in the rat (Quinn et al., 2001, 2003, 2005). The results of these studies now demonstrate that not only has LABORAS™ the ability to detect rat behaviour at a rate and accuracy comparable with conventional observer methods and standard LMA systems, but the capability of the LABORAS™ system to monitor rodent behaviour over a 24 h activity period may prove advantageous in the temporal analysis of pre-clinical models of disease and the assessment of potential therapies.

The present investigation was undertaken to determine the ability of the LABORAS™ system to assess behavioural impairments in mice, following various dopaminergic manipulations. Indeed, the algorithms used by LABORAS™ to detect behaviours in mice are unique and distinct from those used to detect comparable behaviours in rats. An initial pharmacologi-

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cal validation of the LABORAS™ system was therefore carried out using a variety of tool compounds to include: (1) the indirectly acting dopamine (DA) agonist, amphetamine (O'Neill and Shaw, 1999; Starr and Starr, 1986; Vetulani et al., 2001); (2) the D_1/D_2 receptor antagonist, haloperidol (Simon et al., 2000; Starr and Starr, 1986); (3) the D_1 receptor antagonist, SCH23390 (Starr and Starr, 1986); (4) the D_1/D_2 receptor agonist, apomorphine (Di Chiara et al., 1976; Irifune et al., 1995; Starr and Starr, 1986; Thomas and Handley, 1978); (5) the DA precursor L-3,4,dihydroxyphenylalanine (L-DOPA) (Di Chiara et al., 1976; Gronan, 1975). This primary investigation was then followed by an exploration in LABORAS™ of the general locomotor impairments induced in mice following administration of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Systemic administration of MPTP to mice is an established experimental model of idiopathic Parkinson's disease (PD), as the toxin produces a marked depletion of striatal DA, its metabolites and terminals, and destruction of dopaminergic neurons in the pars compacta of the substantia nigra, mimicking the pathological hallmarks of PD (Heikkila et al., 1984; Ricaurte et al., 1987; Sonsalla and Heikkila, 1986). In the clinic such dopaminergic degeneration and loss of DA function manifests itself phenotypically as a 'hypokinetic state', i.e. bradykinesia, tremor, muscular rigidity and a parkinsonian gate (Berardelli et al., 2001; Rascol et al., 2002). Within the preclinical setting however, MPTP dosing protocols in mice are not standardised, resulting in variable degrees of nigro-striatal degeneration and inconsistent reports of functional impairments. Notably spontaneous motor activity is measured, where authors report reductions (Arai et al., 1990; Fredriksson and Archer, 1994; Fredriksson et al., 1997), potentiation (Chia et al., 1996; Rousselet et al., 2003) or no change (Nishi et al., 1991; Schroeder et al., 1997; Willis and Donnan, 1987), in the conventional parameters of LMA. Potential behavioural impairments in an acute model of MPTP-induced nigro-striatal degeneration were therefore investigated using the LABORAS™ system.

2. Materials and methods

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and conformed to GlaxoSmithKline ethical standards.

2.1. Animals

All animals were housed under a 12 h light/dark cycle (lights on 07:00 h).

Dopaminergic manipulations in C57BL6/J mice. Male C57BL6/J mice (25–30 g, Harlan-Olac, UK) were housed in groups of 10 for at least 5 days prior to behavioural testing, with free access to food and water. Mice were placed in a room adjacent to the experimental room on the day of the experimental procedure.

MPTP intoxicated mice. Male C57BL6/J mice (27–32 g, Harlan Olac, UK) were housed individually in a room with an increased temperature (24–25 °C) and allowed to acclimatise for at least 1 week prior to MPTP dosing. The increased temperature was to help maintenance of body temperature after MPTP administration, thereby reducing the adverse effects of hypothermia post-dosing. Prior to dosing, and throughout the remainder of the study, mice were given baby food and softened diet to encourage feeding during the acute post-MPTP adverse effects, and help reduce the risk of dehydration post-dosing. Additional soft bedding and a cardboard house were also provided to encourage nesting.

2.2. *LABORAS*™

The LABORAS™ system (Metris b.v., Hoofddorp, The Netherlands) consists of a triangular shaped sensing platform (carbon fibre plate 700 mm × 700 mm × 1000 × 30 mm, Metris b.v.), positioned on two orthogonally placed force transducers (single point load cells) and a third fixed point attached to a heavy bottom plate (Corian plate 695 mm × 695 mm × 980 × 48 mm, Metris b.v.). The whole structure stands on three spikes, which are adjustable in height and absorb external vibrations. Mice are housed in clear polycarbonate/Makrolon type II cages with wire lid (floor area 352 cm^2, height 14 cm/height to foodhopper 7.5 cm, cage:UNO Roestvaststaal, Zevenaar, The Netherlands, Hopper and Bottle: LabProducts Inc., Seaford, USA), with a sawdust covered floor. One cage is placed directly onto the sensing platform, the upper part of which (including the top, food hopper and drinking bottle) is suspended in a high adjustable frame and is free from the sensing platform. Resultant electrical signals caused by the mechanical vibrations of the movement of the animal are transformed by each force transducer, amplified to a fixed signal range, filtered to eliminate noise, digitised and then stored on a computer. The computer then processes the stored data using several signal analysis techniques to classify the signals into the behavioural categories of feeding, drinking, climbing, immobility and grooming (for details see Quinn et al., 2003; Van de Weerd et al., 2001). The behaviour transitions are determined with a resolution of 0.01 s and the behaviour which dominates is scored. Distance travelled, average and maximum speeds are determined with a resolution of 0.25 s using the animals *XY*-position in LABORAS™ and velocity of movement.

Dopaminergic manipulations—LABORAS™. Mice were habituated to the LABORAS™ cages for 30 min and then injected with amphetamine (0.1–5 mg/kg), haloperidol (0.01–1 mg/kg), SCH23390 (0.03–1 mg/kg), apomorphine (0.05–2 mg/kg), L-DOPA (50–100 mg/kg) in combination with the peripheral dopa decarboxylase inhibitor, benserazide (10 mg/kg) or saline s.c., and immediately placed in a LABORAS™ cage for a 90 min test period. Data was captured as duration (s) and frequency for each behaviour, distance travelled (m), average and maximum speeds (mm/s) and presented as mean ± S.E.M. (n = 7–10/treatment group). Drug effects were analysed by one-way ANOVA followed by Dunnett's test after identification of overall significance.

MPTP-induced nigro-striatal degeneration—LABORAS™. Mice were acutely treated with a single dose of MPTP (30 mg/kg, s.c., neck) or saline and returned to their home cages. Day 6–7 post-MPTP/saline administration, mice were placed

individually into the LABORAS^{TM} cages for a 24 h test period (18:00–18:00 h). On day 8 animals were euthanased by anaesthetic overdose. The brain was removed from each animal, hemisected and the left half immersion fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. The right striatum was dissected out, snap-frozen on cardice and stored at −80 °C for HPLC dopamine neurochemistry. For LABORAS^{TM} data was captured as duration(s) and frequency for each behaviour and presented as mean ± S.E.M. (n = 11–12/treatment group). MPTP effects were compared with saline and analysed by independent t-test.

2.3. Ex-vivo neurochemistry-high performance liquid chromatography (HPLC)

Tissue preparation. Striatum samples were weighed and homogenised in 0.4 M perchloric acid (10 μl/mg tissue), containing sodium metabisulphate (0.1%, w/v), EDTA (0.01%, w/v) and L-cysteine (0.1%, w/v) at a ratio of 100 μl homogenising buffer per mg of striatal tissue (giving a tissue concentration of 0.01 g/ml). The samples were then centrifuged at 10,000 × g at 4 °C for 10 min and the supernatant fraction decanted.

HPLC-ECD (*electrochemical detection*) *analysis*. Aliquots (20 μl) of supernatant were transferred into micro volume glass vials for HPLC-ECD analysis.

A mobile phase composed of 0.07 M KH_2PO_4, containing 1.5 mM sodium octylsulphonate and 0.1 mM EDTA·Na_2, MeOH, tetrahydrofuran (87.5:12:0.5%, w/v) was used at a flow rate of 2.5 ml/min for analysis of samples. Separation was performed using two Chromolith Speedrod columns connected in series (100 mm × 4.6 mm i.d., Lutterworth, UK). Aliquot (10 μl) of sample was injected onto the column. Eluates were detected using a Decade electrochemical detector fitted with a glassy carbon cell (Antec Leyden, The Netherlands) set at +0.65 V versus in situ Ag/AgCl reference electrode. Data were collected using Millennium 32 software (Waters, Milford, MA). The chromatograms were compared with a previously run calibration to identify and quantify components DA, DOPAC (dihydroxyphenylacetic acid), HVA (homovanillic acid), 5-HT (5-hydroxytryptamine), 5-HIAA (hydroxyindolacetic acid) and 3-MT (methoxytyramine). The amount of each monoamine was interpolated from calibration curves derived from prepared standards. Results are expressed in ng/mg of wet weight tissue and presented as mean ± S.E.M. (n = 11–12/treatment group). MPTP effects were compared with saline. Data was analysed for demonstration of normal distribution followed by independent t-test.

2.4. Immunohistochemistry

Wax-embedded blocks including the substantia nigra were sectioned at 5 μm on a rotary microtome. Four sections were prepared for tyrosine hydroxylase (TH) immunohistochemistry from our region of interest at bregma −3.16, according to the mouse brain atlas of Franklin and Paxinos (1997). Sections were microwaved in Tris/borate/ EDTA antigen retrieval buffer, pH 8.3 (Sigma–Aldrich, St. Louis, MO) and microwaved at 1000 W for 3.5 min, 700 W for 2 min, and finally 450 W for 2× 5 min. Sections were left to cool for 20 min, prior to washing in several changes of deionised water and then loaded onto the Dako autostaining machine. Slides were treated with a peroxidase blocking solution for 5 min, antibody serum (Rabbit anti-TH (AB152), Chemicon International, Temecula, CA) diluted to 1:500 in antibody diluent (DakoCytomation, Glostrup, Denmark) for 30 min, LSAB1 biotinylated link (DakoCytomation) for 10 min, LSAB streptavidin HRP 2 (DakoCytomation) for 10 min and finally 3′3′ diaminobenzidine (DAB) chromagen for 10 min. The slides were rinsed with Optimax buffer solution (1:20) after each step, and rinsed with deionised water and running tap water after DAB application. A haematoxylin counterstain was applied to all slides for 3 s, which were then washed in running tap water for 5 min. All slides were dehydrated in ascending Industrial Methylated Spirit concentrations, cleared in Histoclear (National Diagnostics, Hessle Hull, UK), mounted with DPX and coverslipped. Analysis of the sections was carried out on an Olympus BX41 microscope linked to the AnalySIS (Olympus) computer package. Cell counts of the substantia nigra were made for every animal, and the mean of each treatment group taken. The cell bodies included in the substantia nigra were those of TH positive cell bodies situated beyond the medial terminal nucleus of the accessory optic tract. Data are presented as mean ± S.E.M. (n = 11–12/treatment group). MPTP effects were compared with saline. Data was analysed for demonstration of normal distribution followed by independent t-test.

2.5. Drugs

All drug treatments were obtained from Sigma–Aldrich and were administered subcutaneously in 0.9% (w/v) NaCl. Injection volumes were 10 ml/kg for all treatments.

3. Results

3.1. Dopaminergic manipulations—LABORAS^{TM}

3.1.1. Amphetamine-induced hyperactivity

On administration of low dose amphetamine (0.1 mg/kg, s.c.), LABORAS^{TM} detected a significant potentiation of groom duration, decrease in climb duration and immobility frequency and a subsequent increase in immobility duration (Table 1). On administration of amphetamine 1 mg/kg, LABORAS^{TM} also detected a significant decrease in climb duration and increase in immobility duration (Table 1). In addition, administration of amphetamine (5 mg/kg) produced a significant potentiation of LMA duration and frequency (in accordance with an increase in distance travelled, average and maximum speeds) and a corresponding reduction in immobility duration and frequency, groom duration and frequency and climb duration (Table 1), relative to saline-treated controls.

3.1.2. Haloperidol-induced hypoactivity

On administration of haloperidol (0.1–1 mg/kg, s.c.), LABORAS^{TM} detected a dose-related inhibition of LMA

Table 1
Amphetamine-induced effects on locomotor activity, immobility, climbing, grooming, distance travelled, average speed and maximum speed

Dose–response	Saline	Amphetamine (0.1 mg/kg)	Amphetamine (1 mg/kg)	Amphetamine (5 mg/kg)	*F* value (3,28)
Locomotor activity					
Duration (s)	88.2 ± 21.0	63.9 ± 12.9	71.2 ± 7.3	1387.7 ± 156.0**	68.40, $P<0.01$
Frequency	128.6 ± 31.3	83.1 ± 18.9	78.3 ± 8.8	1138.5 ± 58.6**	224.39, $P<0.01$
Immobility					
Duration (s)	1362.4 ± 303.2	2160.6 ± 160.6*	2211.8 ± 212.7*	259.8 ± 50.8**	20.13, $P<0.01$
Frequency	184.1 ± 46.9	85.8 ± 5.8*	264.5 ± 21.4	64.2 ± 7.0**	12.49, $P<0.01$
Climbing					
Duration (s)	33.5 ± 14.3	6.8 ± 4.3*	2.2 ± 2.1*	1.9 ± 1.4*	3.94, $P=0.02$
Frequency	1.3 ± 0.4	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	2.82, $P=0.06$
Grooming					
Duration (s)	171.4 ± 47.9	364.3 ± 41.6**	132.2 ± 47.5	4.8 ± 2.4*	14.08, $P<0.01$
Frequency	23.7 ± 5.3	41.1 ± 5.9	23.5 ± 8.0	1.0 ± 0.3*	8.40, $P<0.01$
Distance travelled (m)	2.6 ± 0.5	2.1 ± 0.4	2.2 ± 0.2	66.3 ± 13.3**	22.85, $P<0.01$
Average speed (mm/s)	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	18.4 ± 3.7**	22.85, $P<0.01$
Maximum speed (mm/s)	148.3 ± 11.0	133.1 ± 11.2	118.6 ± 3.7	191.2 ± 9.9**	10.92, $P<0.01$

Dose–response: amphetamine given s.c. 0 min pre-test. Data are represented as mean ± S.E.M.
* $P<0.05$: significantly different from saline by one-way ANOVA and Dunnett's test.
** $P<0.01$: significantly different from saline by one-way ANOVA and Dunnett's test.

duration (Table 2). In addition, administration of haloperidol (1 mg/kg) produced a significant inhibition of groom duration and frequency and LMA frequency (Table 2). LABORAS™ also detected a significant decrease in distance travelled and average and maximum speeds at a dose of 0.01 mg/kg haloperidol (Table 2).

3.1.3. SCH23390-induced hypoactivity

LABORAS™ detected a significant decrease in LMA duration and frequency, groom duration and frequency, distance travelled and average speed (Table 3), on administration of the D_1 receptor antagonist SCH23390 (0.03–1 mg/kg, s.c.). In addition, LABORAS™ also detected a significant decrease in maximum speed and a subsequent increase in immobility duration on administration of SCH23390 0.1–1 mg/kg (Table 3).

3.1.4. Apomorphine-induced hypoactivity

On administration of apomorphine, LABORAS™ detected a significant potentiation of groom duration and frequency at 0.05 mg/kg, inhibition of groom frequency at 0.5 mg/kg and inhibition of groom duration and frequency at 2 mg/kg (Table 4). In addition, on administration of apomorphine (0.05–2 mg/kg, s.c.), LABORAS™ detected a marked dose-related reduction in LMA duration (Table 4). LABORAS™ also detected a significant reduction in LMA frequency, distance travelled and average speed, with a corresponding increase in immobility frequency

Table 2
Haloperidol-induced effects on locomotor activity, immobility, climbing, grooming, distance travelled, average speed and maximum speed

Dose–response	Saline	Haloperidol (0.01 mg/kg)	Haloperidol (0.1 mg/kg)	Haloperidol (1 mg/kg)	*F* value (3,28)
Locomotor activity					
Duration (s)	60.6 ± 12.2	36.5 ± 4.7	27.5 ± 6.8*	9.2 ± 2.9**	7.95, $P<0.01$
Frequency	70.7 ± 15.5	47.2 ± 3.3	39.8 ± 10.8	11.0 ± 3.3**	5.83, $P=0.01$
Immobility					
Duration (s)	3287.0 ± 359.0	3492.0 ± 421.0	3939.0 ± 367.0	4690.0 ± 421.2	2.36, $P=0.09$
Frequency	208.3 ± 79.4	140.8 ± 82.2	193.0 ± 76.6	149.0 ± 42.7	0.21, $P=0.89$
Climbing					
Duration (s)	62.9 ± 31.6	55.9 ± 31.6	46.8 ± 19.1	15.3 ± 19.2	0.98, $P=0.42$
Frequency	1.8 ± 0.7	1.6 ± 0.4	1.3 ± 0.6	0.5 ± 0.2	1.16, $P=0.34$
Grooming					
Duration (s)	406.8 ± 103.0	413.7 ± 92.5	224.4 ± 45.3	20.9 ± 10.2**	6.46, $P<0.01$
Frequency	38.1 ± 7.3	39.2 ± 5.6	27.0 ± 5.2	4.1 ± 2.0**	9.05, $P<0.01$
Distance travelled (m)	2.5 ± 0.6	0.3 ± 0.1**	1.3 ± 0.4	1.3 ± 0.2	5.82, $P<0.01$
Average speed (mm/s)	0.4 ± 0.1	0.1 ± 0.01**	0.2 ± 0.07	0.2 ± 0.04	5.82, $P<0.01$
Maximum speed (mm/s)	132.0 ± 9.2	96.1 ± 7.9*	129.6 ± 12.1	131.1 ± 9.4	3.18, $P=0.04$

Dose–response: Haloperidol given s.c. 0 min pre-test. Data are represented as mean ± S.E.M.
* $P<0.05$: significantly different from saline by one-way ANOVA and Dunnett's test.
** $P<0.01$: significantly different from saline by one-way ANOVA and Dunnett's test.

Table 3
SCH23390-induced effects on locomotor activity, immobility, climbing, grooming, distance travelled, average speed and maximum speed

Dose–response	Saline	SCH23390 (0.03 mg/kg)	SCH23390 (0.1 mg/kg)	SCH23390 (0.3 mg/kg)	SCH23390 (1 mg/kg)	F value (4,39)
Locomotor activity						
Duration (s)	113.6 ± 18.7	30.8 ± 6.6**	14.1 ± 3.3**	12.6 ± 4.1**	0.9 ± 0.5**	22.48, $P<0.01$
Frequency	171.0 ± 29.0	46.8 ± 12.3**	21.1 ± 5.1**	18.5 ± 6.9**	1.5 ± 0.8**	20.73, $P<0.01$
Immobility						
Duration (s)	3340.1 ± 305.0	4090.0 ± 472.0	4567.0 ± 207.0*	4556.0 ± 377.0*	4786.0 ± 329.0**	3.27, $P=0.02$
Frequency	133.4 ± 33.9	167.7 ± 62.1	184.6 ± 40.7	169.3 ± 88.2	225.4 ± 108	0.18, $P=0.95$
Climbing						
Duration (s)	27.1 ± 10.5	47.1 ± 28.6	5.3 ± 3.5	6.5 ± 4.4	7.1 ± 0.07	2.87, $P=0.03$
Frequency	1.2 ± 0.6	1.3 ± 0.7	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	2.66, $P=0.05$
Grooming						
Duration (s)	344.4 ± 81.4	141.4 ± 18.1**	70.6 ± 13.4**	26.7 ± 10.4**	5.8 ± 2.6**	11.27, $P<0.01$
Frequency	45.2 ± 9.6	23.0 ± 4.1*	11.4 ± 1.7**	5.3 ± 1.8**	2.2 ± 1.1**	11.84, $P<0.01$
Distance travelled (m)	4.5 ± 0.8	1.2 ± 0.3**	0.5 ± 0.1**	0.4 ± 0.1**	0.0 ± 0.0**	19.29, $P<0.01$
Average speed (mm/s)	0.8 ± 0.2	0.2 ± 0.1**	0.1 ± 0.02**	0.1 ± 0.02**	0.0 ± 0.0**	19.26, $P<0.01$
Maximum speed (mm/s)	165.0 ± 9.5	120.8 ± 18.3	99.1 ± 14.3**	97.8 ± 18.5**	31.0 ± 13.4**	11.56, $P<0.01$

Dose–response: SCH23390 given s.c. 0 min pre-test. Data are represented as mean ± S.E.M.
* $P<0.05$: significantly different from saline by one-way ANOVA and Dunnett's test.
** $P<0.01$: significantly different from saline by one-way ANOVA and Dunnett's test.

(Table 4), on administration of apomorphine (0.5–2 mg/kg, s.c.). At the dose of 2 mg/kg, s.c., a significant impairment in mouse climbing frequency, increase in immobility duration and decrease in maximum speed (Table 4) were also noted, compared to saline-treated controls.

3.1.5. L-DOPA-induced hypoactivity

Over the 90-min test period, LABORAS™ detected a significant reduction in LMA duration and frequency (in accordance with a decrease in distance travelled and average speed) and groom duration (Table 5), on administration of L-DOPA (50–200 mg/kg, s.c). LABORAS™ also detected a significant reduction in groom frequency and maximum speed and a subsequent increase in immobility duration (Table 5), on administration of L-DOPA (100–200 mg/kg, s.c.). In addition, LABORAS™ detected a significant increase in immobility frequency on administration of L-DOPA at 50 and 100 mg/kg, however, this effect was abolished at the 200 mg/kg dose (Table 5). A significant impairment in climb frequency was also detected by LABORAS™ at doses of 100 and 200 mg/kg L-DOPA (Table 5).

3.2. MPTP-induced nigro-striatal degeneration

3.2.1. LABORAS™

Mice acutely treated with the dopaminergic neurotoxin MPTP (30 mg/kg, s.c.) and tested day 6–7 post-MPTP admin-

Table 4
Apomorphine-induced effects on locomotor activity, immobility, climbing, grooming, distance travelled, average speed and maximum speed

Dose–response	Saline	Apomorphine (0.05 mg/kg)	Apomorphine (0.5 mg/kg)	Apomorphine (2 mg/kg)	F value (3,24)
Locomotor activity					
Duration (s)	308.0 ± 43.4	187.5 ± 29.7*	53.0 ± 24.4**	4.6 ± 1.1**	19.22, $P<0.01$
Frequency	433.0 ± 59.2	286.0 ± 43.8	97.4 ± 43.7**	7.6 ± 1.3**	17.21, $P<0.01$
Immobility					
Duration (s)	2366.6 ± 356.0	1869.3 ± 342.3	3316.1 ± 585.8	4240.4 ± 226.8*	6.20, $P=0.02$
Frequency	80.8 ± 12.4	115.4 ± 18.1	341.8 ± 28.3**	379.0 ± 29.3**	47.39, $P<0.01$
Climbing					
Duration (s)	246.9 ± 95.2	112.5 ± 82.6	9.7 ± 6.2	5.4 ± 3.4	2.85, $P=0.06$
Frequency	5.3 ± 2.1	3.7 ± 1.2	0.8 ± 0.5	0.3 ± 0.2*	2.97, $P=0.05$
Grooming					
Duration (s)	342.3 ± 54.1	731.2 ± 111.9**	135.7 ± 51.5	53.9 ± 40.1*	17.40, $P<0.01$
Frequency	48.3 ± 6.3	77.2 ± 7.8*	21.5 ± 7.0*	11.8 ± 8.6**	15.15, $P<0.01$
Distance travelled (m)	11.5 ± 1.8	8.9 ± 1.7	2.7 ± 0.9**	0.1 ± 0.0**	13.39, $P<0.01$
Average speed (mm/s)	2.1 ± 0.3	1.6 ± 0.3	0.5 ± 0.1**	0.0 ± 0.0**	13.39, $P<0.01$
Maximum speed (mm/s)	188.5 ± 17.7	198.3 ± 17.9	145.0 ± 19.5	61.6 ± 17.5**	11.17, $P<0.01$

Dose–response: apomorphine given s.c. 0 min pre-test. Data are represented as mean ± standard error of mean
* $P<0.05$: significantly different from saline by one-way ANOVA and Dunnett's test.
** $P<0.01$: significantly different from saline by one-way ANOVA and Dunnett's test.

Table 5
L-DOPA-induced effects on locomotor activity, immobility, climbing, grooming, distance travelled, average speed and maximum speed

Dose–response	Saline	L-DOPA (50 mg/kg)	L-DOPA (100 mg/kg)	L-DOPA (200 mg/kg)	F value (3,32)
Locomotor activity					
Duration (s)	158.8 ± 28.4	52.7 ± 10.2**	12.7 ± 4.2**	7.1 ± 2.6**	23.06, $P<0.01$
Frequency	233.8 ± 42.8	84.3 ± 15.7**	19.5 ± 6.1**	10.6 ± 3.4**	21.82, $P<0.01$
Immobility					
Duration (s)	3058.3 ± 222.0	3115.2 ± 288.2	4450.6 ± 219.0**	4922.7 ± 278**	20.34, $P<0.01$
Frequency	105.1 ± 13.2	343.6 ± 37.4**	318.6 ± 49.3**	107.2 ± 13.7	20.63, $P<0.01$
Climbing					
Duration (s)	51.4 ± 13.0	62.1 ± 33.9	19.9 ± 15.9	0.0 ± 0.0	1.95, $P=0.15$
Frequency	2.5 ± 0.7	1.5 ± 0.7	0.3 ± 0.2*	0.0 ± 0.0**	4.32, $P=0.01$
Grooming					
Duration (s)	446.8 ± 53.4	184.9 ± 40.9**	33.6 ± 17.4**	2.11 ± 1.6**	32.28, $P<0.01$
Frequency	54.0 ± 5.8	36.8 ± 6.47	11.5 ± 4.6**	0.3 ± 0.26**	20.63, $P<0.01$
Distance travelled (m)	6.4 ± 1.4	1.5 ± 0.4**	0.3 ± 0.1**	0.4 ± 0.3**	16.74, $P<0.01$
Average speed (mm/s)	1.2 ± 0.2	0.2 ± 0.07**	0.1 ± 0.0**	0.1 ± 0.1**	16.74, $P<0.01$
Maximum speed (mm/s)	177.1 ± 18.8	145.8 ± 13.7	71.7 ± 22.0**	67.5 ± 22.5**	7.35, $P<0.01$

Dose–response: L-DOPA given s.c. 0 min pre-test. Data are represented as mean ± S.E.M.
* $P<0.05$: significantly different from saline by one-way ANOVA and Dunnett's test.
** $P<0.01$: significantly different from saline by one-way ANOVA and Dunnett's test.

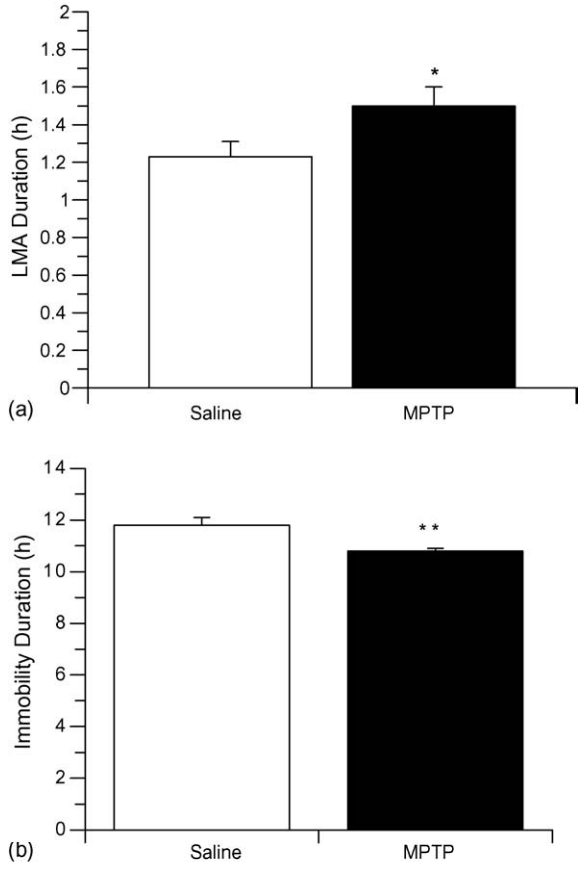

Fig. 1. (a) MPTP-induced increase in LMA duration in mice. (b) MPTP-induced decrease in immobility duration in mice. All data cited as mean ± S.E.M., $n=11$–12/group. * $P<0.05$, ** $P<0.01$: significantly different from vehicle, by independent t-test.

istration in LABORAS™, were detected to have a significant potentiation in LMA duration ($P<0.05$; Fig. 1a), and subsequent decrease in immobility duration ($P<0.01$; Fig. 1b) over the 24 h test period, relative to saline-treated mice. LABORAS™ detected no other significant differences, from saline-treated mice, in the behaviour of MPTP-treated mice (Table 6).

Table 6
MPTP-induced effects on locomotor activity, immobility, climbing, grooming, drinking, eating, distance travelled, average speed and maximum speed, over a 24 h test period

	Saline	MPTP (30 mg/kg)
Locomotor activity		
Frequency	6895.20 ± 457.5	7905.20 ± 483.8
Immobility		
Frequency	1537.30 ± 133.5	1503.70 ± 92.1
Climbing		
Duration (h)	0.60 ± 0.07	0.50 ± 0.07
Frequency	126.40 ± 16.3	123.30 ± 17.8
Grooming		
Duration (h)	1.10 ± 0.08	1.30 ± 0.1
Frequency	604.70 ± 38.6	766.40 ± 87.3
Drinking		
Duration (h)	0.05 ± 0.01	0.07 ± 0.01
Frequency	61.80 ± 11.1	76.30 ± 13.1
Eating		
Duration (h)	0.70 ± 0.06	0.90 ± 0.09
Frequency	623.80 ± 43.8	812.50 ± 89.8
Distance travelled (m)	198.20 ± 15.9	226.30 ± 17.6
Average speed (mm/s)	2.29 ± 0.18	2.62 ± 0.2
Maximum speed (mm/s)	247.90 ± 4.1	262.60 ± 16.9

MPTP given s.c. 6 days pre-test. Data are represented as mean ± S.E.M. MPTP effects are not significantly different from saline by independent t-test.

Table 7

	Saline	MPTP
DOPA	0.913 ± 0.147	1.032 ± 0.181
Dopamine	21.277 ± 1.703	4.777 ± 0.948**
DOPAC	7.120 ± 0.751	4.648 ± 1.456
HVA	6.971 ± 0.354	2.973 ± 0.380**
3MT	1.997 ± 0.241	1.767 ± 1.012
5HTP	0.251 ± 0.170	0.481 ± 0.258
5HT	6.638 ± 0.432	8.050 ± 1.662
5HIAA	3.008 ± 0.142	2.778 ± 0.189

Levels of DA and 5HT, their precursors DOPA and 5HTP and their metabolites DOPAC, HVA, 3MT and 5HTIAA in the striatum at day 7 post-MPTP/saline treatment, as measure by HPLC-ECD. $n = 11–12$/treatment group. All values are expressed as ng/mg of wet weight tissue and presented as mean ± S.E.M.
** $P < 0.01$: significantly different from saline by independent t-test.

3.2.2. Neurochemistry

A marked reduction of dopamine levels in the striatum of MPTP-treated mice was observed (78%, $P < 0.01$, Table 7), compared with saline-treated controls. The level of the dopamine metabolite HVA was also reduced in the MPTP-treated group (57% HVA, $P < 0.01$, Table 7). Dopamine turnover (corresponding to the ratio of dopamine metabolites to dopamine) was increased in the MPTP-treated group by 140%.

3.2.3. Immunohistochemistry

A 48% reduction in TH-immunoreactivity was observed in the substantia nigra of MPTP-treated mice, 8 days after MPTP intoxication ($P < 0.01$; Fig. 2a and b).

4. Discussion

4.1. Amphetamine-induced hyperactivity in mice

The increased LMA, detected by LABORAS^{TM} on administration of amphetamine 5 mg/kg, is comparable with published data in which doses of amphetamine at 2 mg/kg or above were required to induce a significant potentiation of LMA duration above vehicle controls, in standard LMA boxes (O'Neill and Shaw, 1999; Starr and Starr, 1986; Vetulani et al., 2001). In addition, the high dose inhibition (amphetamine, 5 mg/kg) of groom activity is also comparable with a previous investigation, in which doses of amphetamine at 2 mg/kg or above were observed to significantly decrease groom duration in mice (Starr and Starr, 1986). Starr and Starr (1986), also reported that doses of amphetamine in the range 0.1–0.5 mg/kg were observed to increase groom frequency. In the present investigation, administration of amphetamine at a dose of 0.1 mg/kg significantly increased groom duration but not frequency.

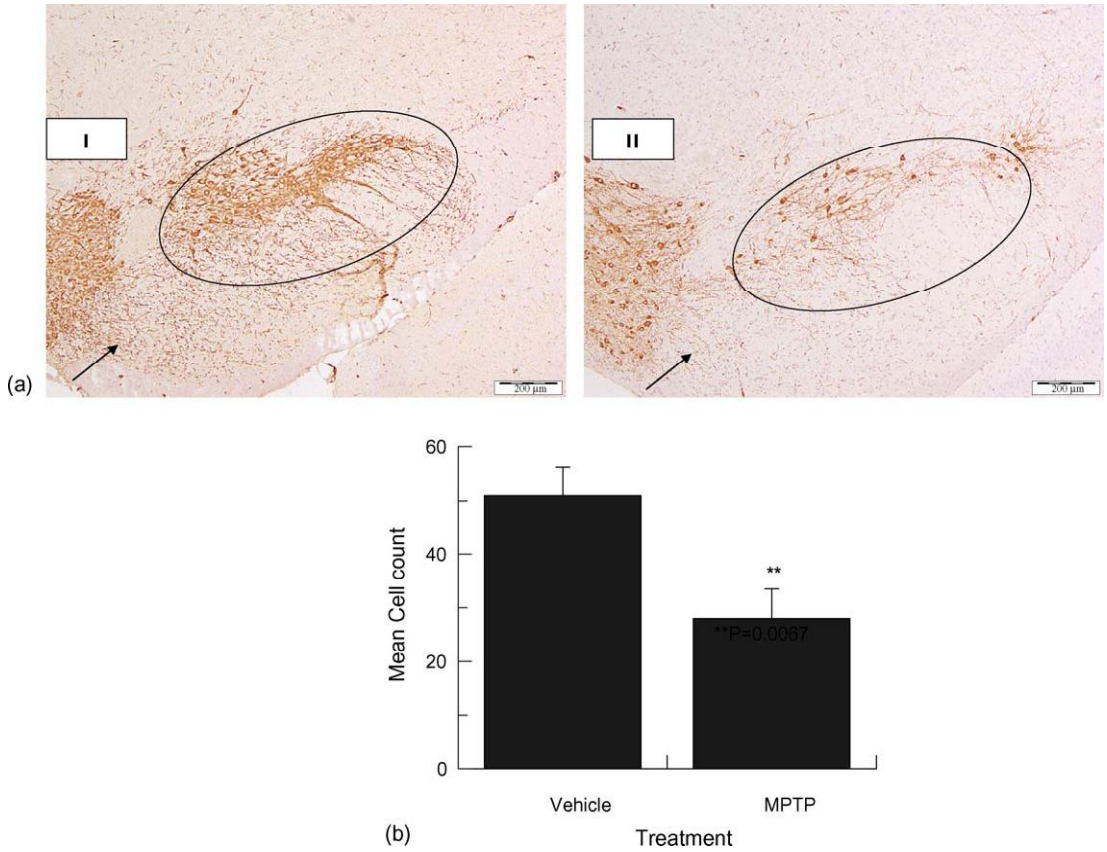

Fig. 2. (a) Photomicrograph of MPTP-induced TH-positive cell decrease in the substantia nigra: (I) saline treated animal and (II) MPTP treated animal. (b) MPTP-induced decrease in TH-positive cell counts in the substantia nigra. All data cited as mean ± S.E.M., $n = 11–12$/group. ** $P < 0.01$: significantly different from vehicle, by independent t-test.

4.2. Haloperidol-induced hypoactivity

In LABORAS™, the inhibition of LMA duration, produced by haloperidol 0.1–1 mg/kg, is comparable with studies in which significant decreases in motor activity were induced with haloperidol from 0.1 mg/kg (Simon et al., 2000; Starr and Starr, 1986). In addition, the inhibition of groom activity, produced by haloperidol 1 mg/kg, is supported by data in which significant impairments in groom duration were observed from 0.2 mg/kg (Starr and Starr, 1986; Vasse and Protais, 1988).

4.3. SCH23390-induced hypoactivity

The significant decrease in LMA and groom activity, detected by LABORAS™ on administration of the D_1 receptor antagonist SCH23390 (0.03–1 mg/kg), is comparable with studies carried out by Starr and Starr (1986). These authors observed that mice treated with SCH23390 (0.001–0.05 mg/kg) displayed significantly impaired LMA (proximity sensor boxes), and decreased groom duration (visual observation). These data are further supported by Vasse and Protais (1988), who reported a dose-related decrease in groom activity on administration of SCH23390. The stereotypical profile of SCH23390, observed in the present investigation is further supported by published findings in which doses in the range of 0.1–1 mg/kg have been shown to produce catalepsy in mice (Ushijima et al., 1995).

4.4. Apomorphine-induced hypoactivity

The inhibition of LMA duration (0.05–2 mg/kg) and LMA frequency (0.5–2 mg/kg) produced by apomorphine, as assessed by LABORAS™, is comparable with published findings in which low-dose apomorphine administration has been reported to impair motor activity, as a result of dopamine autoreceptor activation and the inhibition of dopamine synthesis (Di Chiara et al., 1976; Irifune et al., 1995; Starr and Starr, 1986; Thomas and Handley, 1978). However, the apomorphine-induced (2 mg/kg) decrease in LMA, registered by LABORAS™, is in contrast to previously reported investigations in which apomorphine in the dose range 1–5 mg/kg is reported to potentiate LMA, as a result of the occupancy of postsynaptic dopamine receptors and increased dopamine neurotransmitter release (Irifune et al., 1995; Matsumoto et al., 1990; Starr and Starr, 1986; Thomas and Handley, 1978). Such differences in reported findings may be caused by the different sensitivities of mouse strains to apomorphine. Indeed, the biphasic activity response to apomorphine was observed in ddy and albino mouse strains (Irifune et al., 1995; Matsumoto et al., 1990; Starr and Starr, 1986). Moreover, it has been observed that C57BL6/J mice treated in the dose range 1–8 mg/kg, did produce a bi-phasic activity profile, but mice of this strain administered with a 1 mg/kg dose of apomorphine still displayed a suppressed LMA, and a dose of 8 mg/kg was required to significantly elevate the motor activity of these animals (Vetulani et al., 1982). This is further supported by observations that C57BL/6 mice treated with apomorphine (0.5–2.5 mg/kg), were significantly impaired in movement initiation and their ability to remain on an accelerating rotarod (Rozas et al., 1998).

The significant inhibition of groom duration (2 mg/kg) and frequency (0.5–2 mg/kg), as detected by LABORAS™ on administration of apomorphine, is comparable with data in which doses of apomorphine (0.2–6 mg/kg) were observed to decrease groom activity (Starr and Starr, 1986; Vasse and Protais, 1988).

4.5. L-DOPA-induced hypoactivity

In LABORAS™, the significant decrease in LMA duration and frequency, produced by L-DOPA (50–200 mg/kg) in combination with benserazide (10 mg/kg), is comparable with published data in which doses of L-DOPA in the range 25–250 mg/kg (in the presence of a decarboxylase inhibitor) have been shown to significantly impair motor activity, when assessed in standard motility cages (Di Chiara et al., 1976; Gronan, 1975). As with apomorphine, the activity of L-DOPA in mice is reported to be biphasic and therefore such inhibition of spontaneous LMA is concluded to be the result of dopamine autoreceptor occupancy and a decreased dopaminergic output. In contrast to these findings however, doses of L-DOPA that would be expected to produce hypoactivity, have been reported to potentiate the motor activity of mice (Johnson et al., 1976; Stromberg and Svensson, 1975). Again, as discussed with apomorphine, such variations in reported findings may be caused by the different sensitivities of mouse strains to L-DOPA. However, it should be noted that the time-frame of the experimental investigation has also been highlighted to influence the activity readouts produced by L-DOPA, and in some investigations a significant potentiation of LMA has been reported to take place post-hypoactivity (Johnson et al., 1976; Stromberg and Svensson, 1975). Moreover, in recent years it has been demonstrated that decarboxylase inhibitors, when administered in high enough doses are capable of central penetration, influencing the extracellular levels of striatal dopamine (Jonkers et al., 2001).

4.6. MPTP-induced nigro-striatal degeneration

It is difficult to compare data from the present study with that of other investigative groups, as MPTP dosing protocols are not standardised and result in variable degrees of nigro-striatal degeneration. The potentiation in LMA, over a 24 h test period, as detected by LABORAS™ day 6–7 post-acute MPTP administration (30 mg/kg), is in agreement with published findings in which C57BL/6 mice treated with sub-chronic MPTP dosing regimens (cumulative doses between 350 and 540 mg/kg, resulting in striatal dopamine depletions of 80–90%) are reported to be significantly hyperactive, when compared with saline-treated controls (Chia et al., 1996; Rousselet et al., 2003). In contrast, these investigators also observed that mice administered with acute (60 mg/kg, striatal dopamine depletion of ~90%), or subchronic MPTP dosing protocols (cumulative doses 200–300 mg/kg, striatal dopamine depletions of 72–74%), were not hyperactive. However, it should be highlighted that these investigators measured the locomotor activity of MPTP-

treated animals for test periods of up to 1 h in standard LMA boxes, in comparison to the 24 h test period of the present investigation in LABORAS^{TM}. More subtle changes in LMA may therefore not have been observed under the conditions of this shorter experimental test period. Indeed, MPTP-treated animals were not detected to be significantly hyperactive in the first hour of LABORAS^{TM} experimentation (mean ± S.E.M.: saline, 378.2 ± 27.8 s; MPTP, 458.1 ± 36.3 s).

The marked loss of striatal dopamine content (78%) in contrast to dopaminergic cell bodies (48%), observed in this model, is also comparable with previous investigations and agrees with the large volume of published evidence that concludes dopaminergic terminals to be significantly more vulnerable to insult by the MPTP neurotoxin (Arai et al., 1990; Rousselet et al., 2003; Sundstrom et al., 1987). Indeed, the hyperactivity observed in the present MPTP model, is the probable (functional) outcome of compensatory mechanisms initiated in surviving dopaminergic neurons, in response to this partial denervation of the nigro-striatal pathway (Bezard and Gross, 1998). Moreover, dopamine turnover was noted to be significantly potentiated post-MPTP administration in the present investigation and previously, neurotoxic lesioning, using an acute model of MPTP, has been shown to produce post-synaptic dopamine receptor upregulation and supersensitivity (Schroeder et al., 1997). However, we cannot conclude that this hyperactivity is solely the result of compensatory mechanisms in the dopaminergic transmission of the nigro-striatal pathway, as other neurochemical alterations in the striatum or other brain regions could contribute to the locomotor response. Certainly, changes in other catecholamine containing systems have been observed in acute, chronic and subchronic dosing regimens of MPTP administration (Di Chiara et al., 1976; Ogawa et al., 1987; Rousselet et al., 2003; Sedelis et al., 2000; Willis and Donnan, 1987). In addition, it should also be highlighted that the hyperactivity registered by LABORAS^{TM}, on administration of MPTP, is in contrast to some previously reported investigations in which acute, subchronic, and chronic dosing schedules of this neurotoxin have been shown to produce no change, or a decrease in the spontaneous LMA of C57BL/6 mice (Arai et al., 1990; Fredriksson and Archer, 1994; Fredriksson et al., 1997; Nishi et al., 1991; Schroeder et al., 1997; Willis and Donnan, 1987).

In conclusion the data described in the present study provides evidence that LABORAS^{TM} is able to sensitively detect pharmacologically induced changes in a range of behaviours in the mouse and that functional deficits following an acute MPTP dosing schedule in mice, can be effectively registered using this automated system.

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