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LABORAS™: Initial pharmacological validation of a system allowing continuous monitoring of laboratory rodent behaviour

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Abstract

A newly developed apparatus for automated behavioural analysis, Laboratory Animal Behaviour Observation, Registration and Analysis System (LABORAS™), has been further validated with respect to the ability of the system to detect the pharmacodynamic effects of standard pharmacological tools. Data were obtained from rats administered with mCPP (reversal with SB242084), 8-OH-DPAT (reversal with WAY100635), amphetamine (reversal with haloperidol) and angiotensin, with the focus on locomotor activity, feeding and drinking behaviours. The data captured and analysed by LABORAS™, suggests that the automated system is able to detect pharmacologically induced changes in behaviour, reliably and efficiently, with a significant reduction in the number of animals required, and reduced operator input.

Keywords: Automatic behaviour registration; Behavioural analysis; mCPP; 8-OH-DPAT; Amphetamine; Angiotensin II; Rats

1. Introduction

Previously, locomotor activity (LMA), feeding and drinking behaviours in mice and rats have been monitored using standard LMA beam-break boxes (Arnt, 1995; Kennett et al., 1997; Duxon et al., 2000) and conventional observer methods to include stopclocks and weigh machines (Kennett et al., 1997; Jones et al., 2000). Recently, an automated behavioural classification system, Laboratory Animal Behaviour Observation, Registration and Analysis System (LABORAS™), has been developed to register the duration and frequency of feeding, drinking, rearing, climbing, immobility, 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 (Bulthuis et al., 1998; Schlingmann et al., 1998).

Previously, baseline behavioural data from a variety of pharmacologically naive 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 address potential improvements highlighted by these initial studies, with respect to the behavioural categories of eating, drinking and grooming (Quinn et al., 2001). The results of these studies now demonstrate a 90–95% correlation of LABORAS™ scores with those of human observers.

The following studies were undertaken to assess the ability of LABORAS™ to detect pharmacologically driven behaviours in the rat: (1) mCPP-induced hypolocomotion and hypophagia, and reversal with the selective $5HT_{2C}$ receptor antagonist SB242084 (Kennett and Curzon, 1988; Kennett, 1993; Kennett et al., 1994, 1997); (2) 8-OH-DPAT-induced hyperlocomotion, and reversal with the selective $5HT_{1A}$ receptor antagonist

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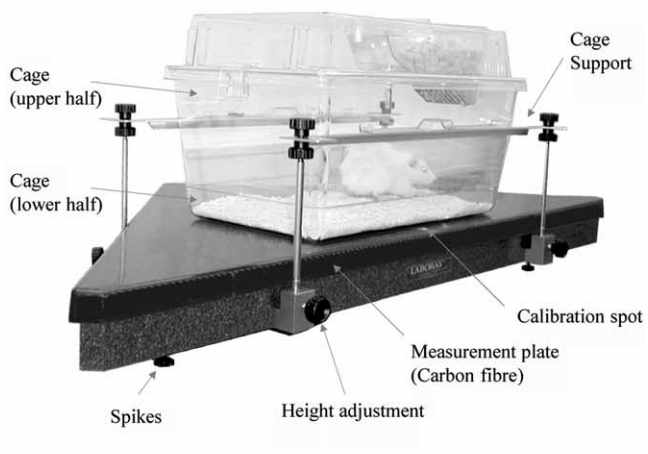
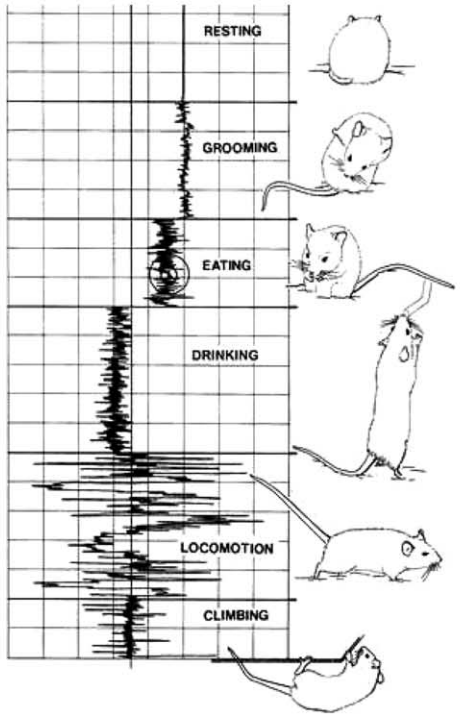


(a)



(b)

Fig. 1. (a) The LABORAS™ behavioural registration system. Sensor platform with cage. (b) Vibration signatures of different rat behaviours as detected by LABORAS, and demonstrated here by chart recorder display.

WAY100635 (Tricklebank et al., 1984; Forster et al., 1995; Fletcher et al., 1996; Duxon et al., 2000); (3) D-amphetamine-induced hyperactivity and reversal with the classical antipsychotic haloperidol (Arnt, 1992, 1995), and finally, (4) drinking induced by intracerebroventricular (i.c.v.) angiotensin-II administration (Simpson et al., 1978).

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

Male Sprague–Dawley rats (250–300 g, Charles River) were housed in groups of six for at least 5 days prior to behavioural testing, under a 12 h light/dark cycle (lights on 07:00) with free access to food and water. Rats were placed in a room adjacent to the experimental room on the day of the experimental procedure.

2.2. LABORAS™

The LABORAS™ system (Metris b.v., Hoofddorp, The Netherlands. Fig. 1a) consists of a triangular shaped sensing platform (Carbon Fibre Plate 700 × 700 × 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 × 695 × 980 × 48 mm, Metris b.v.). The whole structure stands on three spikes, which are adjustable in height and absorb external vibrations. Rats are housed in clear polycarbonate/Makrolon type III cages (floor area 840 cm^2, height 25 cm/height to food hopper 15 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, rearing, climbing, immobility, LMA and grooming (for details see Van de Weerd et al., 2001). The behaviour which dominates is scored. Positional and vibrational parameters are used in the behaviour classification algorithms are indicated in Table 1, vibration patterns corresponding to individual behaviours are represented pictorially in Fig. 1b.

2.3. mCPP-induced hypoactivity

Rats were injected with mCPP (0.5–7 mg/kg, doses chosen from Kennett and Curzon, 1988) or saline i.p., 20 min prior to being placed in a LABORAS™ cage for a 10-min test period.

Table 1
Parameters used in the behavioural classification algorithms

	Locomotion	Immobility	Rearing	Grooming	Drinking	Eating
Centre of gravity						
Absolute location			v		v	v
Displacement	v		v			
Speed	v	v	v	v		
Acceleration	v					
Vibration						
Pattern			v	v	v	v
Frequency				v	v	v
Amplitude		v	v	v	v	v

2.4. SB242084 reversal of mCPP-induced hypoactivity

Rats were administered with SB242084 (0.03–1 mg/kg, doses chosen from Kennett et al., 1997) or vehicle i.p., 30 min prior to testing, and mCPP (7 mg/kg, dose producing almost complete inhibition of LMA, as determined in previous dose–response evaluation to mCPP) or saline i.p., 20 min prior to a 10-min assessment period in individual LABORAS™ cages.

2.5. SB242084 reversal of mCPP-induced hypophagia

Rats were food-deprived for 23 h, then orally dosed with antagonist (SB242084, 2 or 6 mg/kg, doses chosen from Kennett et al., 1997) or vehicle and returned to their home cages. Forty minutes later rats were given mCPP (5 mg/kg, dose chosen from Kennett et al., 1997) or saline i.p., and again returned to their home cages. After a further 20 min they were placed into the LABORAS™ cages and their eating activity (duration and frequency) was recorded for 1 h.

2.6. 8-OH-DPAT-induced hyperactivity

Animals were injected with 8-OH-DPAT (0.01–1 mg/kg, s.c., neck, doses chosen from Tricklebank et al., 1984) or saline and immediately placed into LABORAS™ cages for 30-min behavioural assessment.

2.7. WAY100635 reversal of 8-OH-DPAT-induced hyperlocomotion

Animals were allocated to receive either a single pretreatment of WAY100635 (0.001–0.1 mg/kg, s.c., flank, doses chosen from Duxon et al., 2000) or vehicle (sterilised water), 20 min prior to 8-OH-DPAT (0.3 mg/kg, s.c., neck, dose producing a significant potentiation of LMA, as determined in previous dose–response evaluation to 8-OH-DPAT) or saline and immediately placed into individual LABORAS™ cages for a 30-min test period.

2.8. D-Amphetamine-induced hyperactivity

Rats were habituated to the LABORAS™ cages for 30 min and then dosed with D-amphetamine (0.12–4 mg/kg, s.c., flank, doses chosen from Arnt, 1995) or saline. Their behaviour was recorded for 1 h in LABORAS™.

2.9. Haloperidol reversal of D-amphetamine-induced hyperactivity

Rats were administered with haloperidol (0.16–5 mg/kg, doses chosen from Arnt, 1995) or vehicle p.o. and 1 h later habituated to the LABORAS™ cages for 30 min. Habituated rats were then dosed with D-amphetamine (0.8 mg/kg, s.c., flank, dose producing a significant

Table 2
mCPP-induced hypolocomotion and reversal with SB242084

	LMA	
	Duration (s)	Frequency
Dose–response		
Saline	46.6±16.5	44.1±5.5
mCPP 0.5 mg/kg	35.3±6.1	32.5±5.1
mCPP 1 mg/kg	22.7±6.1**	24.8±6.1*
mCPP 3 mg/kg	6.2±1.8**	1.2±0.6**
mCPP 7 mg/kg	1.2±0.8**	1.2±0.6**
Reversal		
Vehicle+saline	43.5±5.8	37.9±4.1
Vehicle+mCPP (7 mg/kg)	0.0**	0.8±0.8**
SB242084 0.03 (mg/kg)+mCPP	0.1±0.1	0.2±0.2
SB242084 0.1 (mg/kg)+mCPP	9.5±4.2	8.0±4.7
SB242084 0.3 (mg/kg)+mCPP	19.3±4.1	16.2±3.5
SB242084 1 (mg/kg)+mCPP	69.9±15.3^{++}	48.3±8.0^{++}
Vehicle+saline	64.4±8.9	57.4±7.7
SB242084 1 (mg/kg)+saline	73.4±11.3	77.1±12.7

Dose–response: mCPP given i.p. 20-min pre-test, $n=8$–10. *Reversal:* SB-242084 given i.p. 30-min pre-test, mCPP given i.p. 20-min pre-test, $n=7$–8. Data are represented as mean±S.E.M. *$P<0.05$, **$P<0.01$, significantly different from saline or vehicle/saline; $^{++}P<0.01$, significantly different from vehicle/mCPP treated group by one-way ANOVA and Dunnett's test. *Abbreviations:* LMA, locomotor activity.

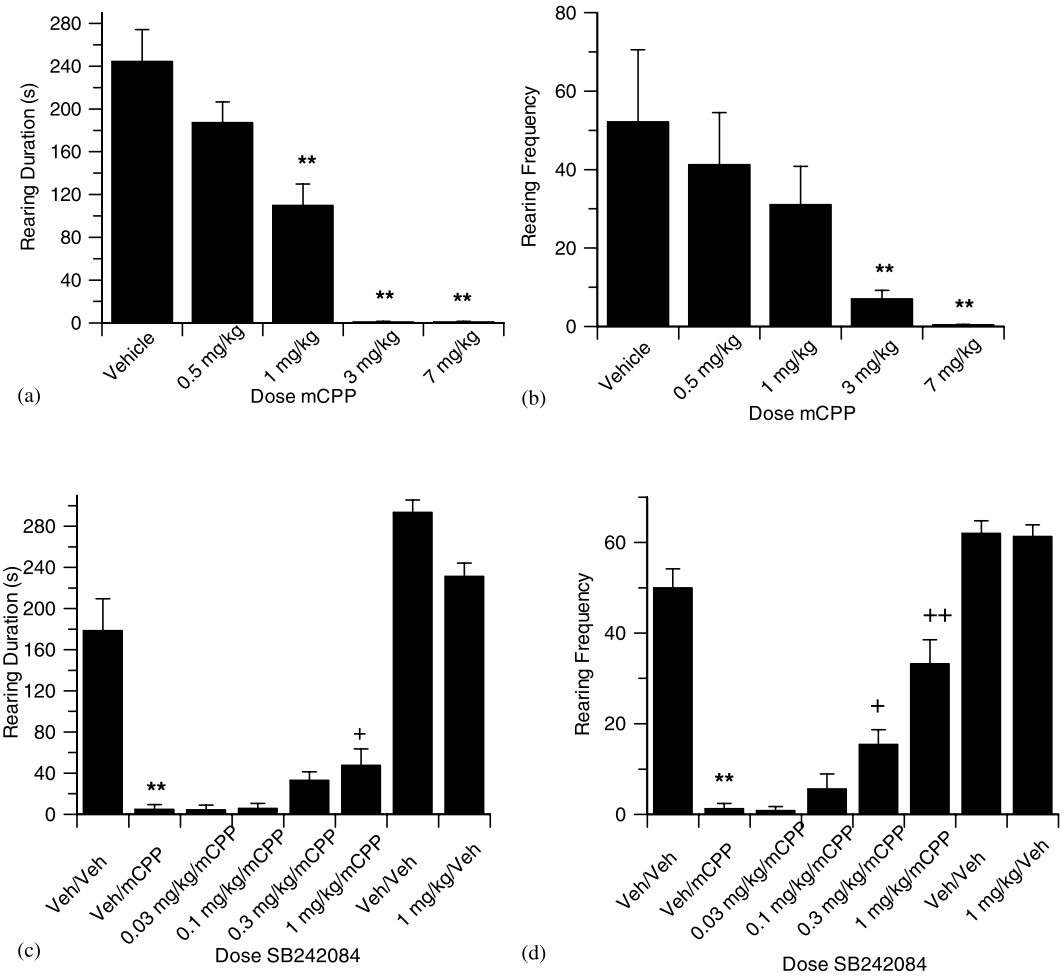


Fig. 2. (a) mCPP-induced decrease in rearing duration in rats. (b) mCPP-induced decrease in rearing frequency in rats. All data cited as mean ± S.E.M., $n = 8$–10/group. $^{**}P < 0.01$, significantly different from vehicle, by Dunnett's test and one-way ANOVA. (c) Effect of SB242084 on mCPP-induced decrease in rearing duration in rats. (d) Effect of SB242084 on mCPP-induced decrease in rearing frequency in rats. All data cited as mean ± S.E.M., $n = 7$–8/group, mCPP administered at 7 mg/kg i.p. $^{**}P < 0.01$, significantly different from vehicle/vehicle treated group, $^{+}P < 0.05$, $^{++}P < 0.01$, significantly different from vehicle/mCPP treated group by Dunnett's test and one-way ANOVA.

potentiation of LMA, as determined in a previous dose–response evaluation to D-amphetamine) or saline and behaviour monitored for 1 h in LABORAS™.

2.10. Angiotensin-II-induced drinking

2.10.1. Animals, surgery and behavioural testing

Male Sprague–Dawley rats (280–300 g at the time of surgery; Charles River), were surgically implanted under gaseous anaesthesia (isoflurane) with an i.c.v. cannula directed towards either the left or right lateral ventricle (±1.6 mm AP, ±0.8 mm ML, −4.1 mm from bregma/skull surface) according to Paxinos and Watson (1986). During recovery animals were placed in a temperature controlled environment (20 ± 1 °C) with free access to food and water. All rats were individually housed for the duration of the study. Seven days post-surgery, animals were administered with either angiotensin-II (100 ng) or vehicle, i.c.v. (see Simpson et al., 1978) and immediately placed into LABORAS™ cages for drinking evaluation over a 30-min test period.

2.11. Reagents

SB242084, (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indolene) and WAY100635, (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide) oxolate), were synthesised by the Department of Medicinal Chemistry, GlaxoSmithKline (Harlow, UK). SB-242084 was either given i.p. in 0.9% w/v NaCl containing 8% (w/v) hydroxypropyl-β-cyclodextrin (Encapsin, Janseen, Biotech N.V., Olen, Belgium) by weight and 25 mM citric acid, or orally as a suspension after grinding (using a pestle and mortar) into a 1% methyl cellulose solution containing a drop of BRIJ 35 (Sigma,

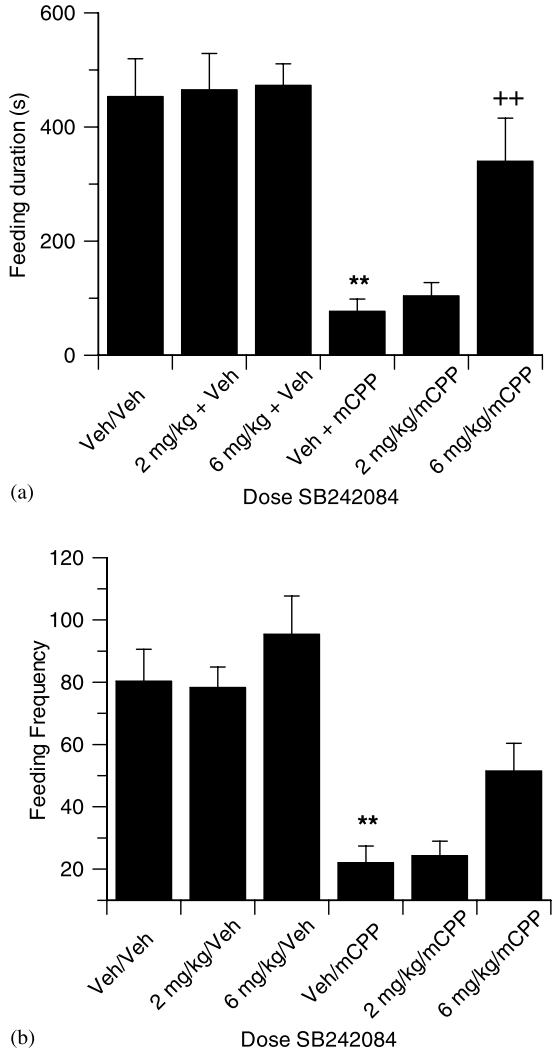


Fig. 3. (a) Effect of SB242084 on mCPP-induced decrease in feeding duration in rats. (b) Effect of SB242084 on mCPP-induced decrease in feeding frequency in rats. All data cited as mean ± S.E.M., $n = 10$/group, mCPP administered at 5 mg/kg i.p. $^{**}P < 0.01$, significantly different from vehicle/vehicle treated group, $^{++}P < 0.01$, significantly different from vehicle /mCPP group, by Dunnett's and one-way ANOVA.

UK). Injection volumes were 2 ml/kg for all SB-242084 treatments. WAY100635 was given subcutaneously in distilled water, 1 ml/kg. m-CPP (m-chlorophenylpiperazine) and amphetamine hydrochloride (Sigma) were dissolved in 0.9% w/v NaCl and injected in a 1 ml/kg volume. 8-OH-DPAT, ((±)-8-hydroxy-2-(dipropylamino) tetralin (hydrobromide salt; Semat) was administered subcutaneously in 0.9% w/v NaCl to give a constant injection volume of 1 ml/kg. Haloperidol (Sigma) was dissolved in an equal weight of tartaric acid and distilled water with a 2 ml/kg dose volume. Angiotensin-II, (acetate salt, human; Sigma) was dissolved in saline to give a constant injection volume of 100 ng/5 μl.

Table 3
8-OH-DPAT-induced inhibition of rearing and reversal with WAY100635

	Rearing	
	Duration (s)	Frequency
Dose–response		
Vehicle	352.6+71.7	84.6+16.9
8-OH-DPAT 0.01 mg/kg	304.5+27.6	73.2+12.0
8-OH-DPAT 0.1 mg/kg	137.2+17.1**	66.4+8.3
8-OH-DPAT 1 mg/kg	83.8+9.0**	53.8+6.7
Reversal		
Vehicle+saline	357.8+39.4	98.7+10.9
Vehicle+DPAT (0.3 mg/kg)	157.3+17.7**	80.7+8.6**
WAY 0.001+8-OH-DPAT	144.0+17.8	70.5+6.9
WAY 0.003+8-OH-DPAT	214.9+36.0	75.9+8.9
WAY 0.01+8-OH-DPAT	192.7+22.9	64.8+4.9
WAY 0.1+8-OH-DPAT	237.9+19.8^{++}	66.2+3.4
Vehicle+saline	333.58±43.5	81.5±9.9
WAY 0.1+saline	462.7±63.1	109.1±14.5

Dose response: 8-OH-DPAT given s.c. at test time, $n = 7–8$; *Reversal:* WAY100635 given s.c. 30-min pre-test, 8-OH-DPAT given s.c. at test time, $n = 10$. Data are expressed as mean ± S.E.M. $^{**}P < 0.01$, significantly different from vehicle or vehicle/saline, $^{++}P < 0.01$ from vehicle/8-OH-DPAT treated group by one-way ANOVA and Dunnett's test.

2.12. Data analysis

Data were captured as duration (seconds) and frequency for each behaviour and presented as mean ± S.E.M. for each treatment group. Drug effects were analysed by one-way ANOVA followed by Dunnett's test after identification of overall significance. The dose of SB-242084 producing 50% disinhibition of mCPP (ID_{50}) was also estimated by the four parameter-logistic function using the iterative curve fitting software ALLFIT (DeLean et al., 1978).

3. Results

3.1. mCPP-induced hypoactivity

LABORAS™ detected a marked dose-dependent reduction in LMA duration ($F(4, 43) = 15.8$, $P < 0.01$; Table 2) and frequency ($F(4, 43) = 16.0$, $P < 0.01$; Table 2), and rearing duration ($F(4, 43) = 34.36$, $P < 0.01$; Fig. 2a) and frequency ($F(4, 43) = 32.39$, $P < 0.01$; Fig. 2b), on administration of mCPP (0.5–7 mg/kg, i.p.).

3.2. Effect of SB242084 on mCPP-induced hypoactivity

On pretreatment with SB242084 (0.03–1 mg/kg, i.p.), LABORAS™ detected a significant dose-related inhibition of the mCPP (7 mg/kg, i.p.) induced reduction in LMA duration ($F(5, 40) = 12.59$, $P < 0.01$; Table 2) and

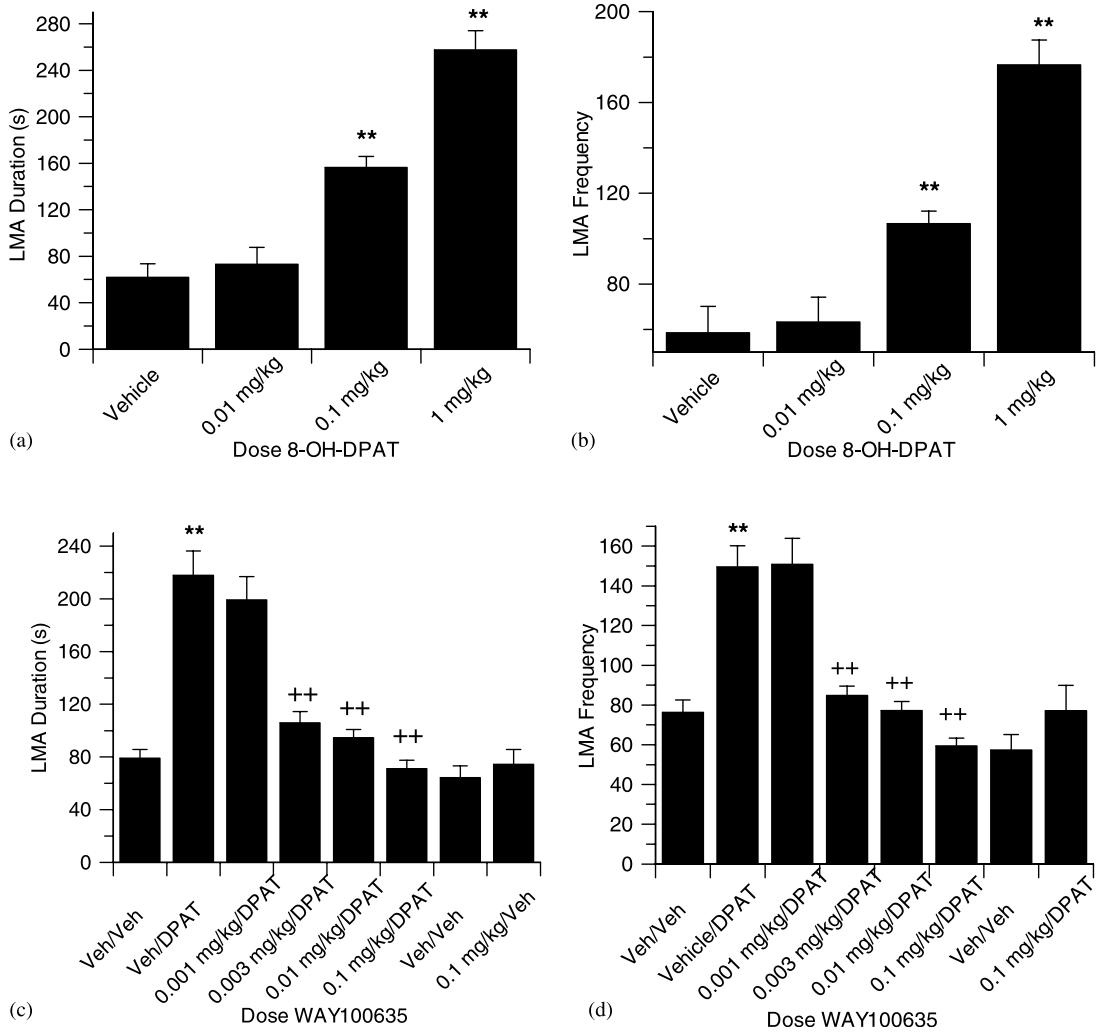


Fig. 4. (a) 8-OH-DPAT-induced increase in locomotor duration in rats. (b) 8-OH-DPAT-induced increase in locomotor frequency in rats. All data cited as mean ± S.E.M., $n = 7$–8/group. $^{**}P < 0.01$, significantly different from vehicle, by Dunnett's and one-way ANOVA. (c) Effect of WAY100635 on 8-OH-DPAT-induced increase in locomotor duration in rats. (d) Effect of WAY100635 on 8-OH-DPAT-induced increase in locomotor frequency in rats. All data cited as mean ± S.E.M., 8-OH-DPAT administered at 0.3 mg/kg s.c. $^{**}P < 0.01$, significantly different from vehicle/vehicle group; $^{++}P < 0.01$, significantly different from vehicle/8-OH-DPAT group by Dunnett's and one-way ANOVA.

frequency ($F(5, 40) = 19.37$, $P < 0.01$; Table 2) and rearing duration ($F(5, 40) = 18.79$, $P < 0.01$; Fig. 2c) and frequency ($F(5, 40) = 32.45$, $P < 0.01$; Fig. 2d). The dose of SB242084 producing 50% inhibition of the action of mCPP was estimated at 0.34 ± 0.14 mg/kg for LMA frequency and 0.6 ± 0.02 mg/kg for rearing frequency.

3.3. Effect of SB242084 on mCPP-induced hypophagia

Over the 1-h test period, LABORAS™ detected a significant reduction in the feeding activity of 23 h food-deprived rats administered mCPP (5 mg/kg, $P < 0.01$; Fig. 3a and b). SB242084 significantly reversed the duration of the hypophagic response to mCPP at 6 mg/kg ($F(5, 54) = 12.16$, $P < 0.01$; Fig. 3a). There was no effect of SB242084 on feeding activity when administered alone.

3.4. 8-OH-DPAT-induced hyperlocomotion

Administration of 8-OH-DPAT (0.01–1 mg/kg, s.c.) immediately prior to assessment in LABORAS™ produced a significant dose-dependent increase in LMA duration ($F(3, 28) = 6.83$, $P < 0.01$; Fig. 4a) and frequency ($F(3, 28) = 29.48$, $P < 0.01$; Fig. 4b), and a significant decrease in rearing duration ($F(3, 28) = 9.57$, $P < 0.01$; Table 3), relative to vehicle treated controls.

3.5. Effect of WAY100635 on 8-OH-DPAT induced hyperlocomotion

On pretreatment of rats with WAY100635 (0.001–0.1 mg/kg, s.c.), LABORAS™ detected a significant dose-dependent inhibition of 8-OH-DPAT (0.3 mg/kg, s.c.) induced potentiation of LMA duration ($F(5, 54) =$

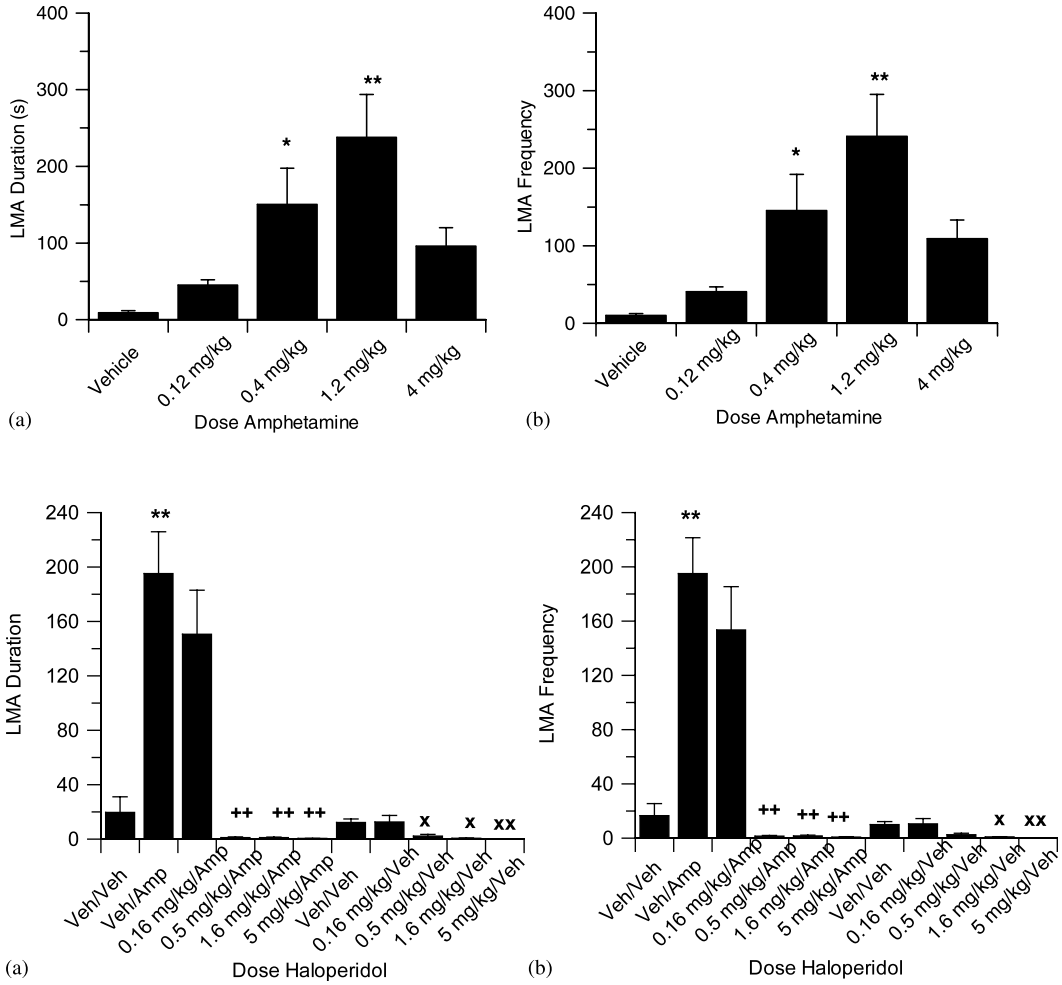


Fig. 5. (a) Amphetamine-induced increase in locomotor duration in rats. (b) Amphetamine-induced increase in locomotor frequency in rats. All data cited as mean ± S.E.M., $n = 9$/group. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from vehicle, by Dunnett's test and one-way ANOVA. (c) Effect of haloperidol on amphetamine-induced increase in locomotor duration in rats. (d) Effect of haloperidol on amphetamine-induced increase in locomotor frequency in rats. All data cited as mean ± S.E.M., $n = 8$–11/group, amphetamine administered at 0.8 mg/kg s.c. $^{**}P < 0.01$, significantly different from vehicle/vehicle treated group, $^{++}P < 0.01$, significantly different from vehicle/amphetamine treated group by Dunnett's test and one-way ANOVA, $^{\times}P < 0.05$, $^{\times\times}P < 0.01$, significantly different from second vehicle/vehicle group, by Dunnett's test and one-way ANOVA.

28.63, $P < 0.01$; Fig. 4c) and frequency ($F(5, 54) = 25.38$, $P < 0.01$; Fig. 4d) and a significant reversal of 8-OH-DPAT-induced inhibition of rearing duration (0.1 mg/kg; $F(5, 54) = 8.8$, $P < 0.01$; Table 3).

3.6. D-Amphetamine-induced hyperactivity

LABORAS™ detected a dose-dependent increase in LMA duration ($F(4, 43) = 6.89$, $P < 0.01$; Fig. 5a) and frequency ($F(4, 43) = 7.39$, $P < 0.01$; Fig. 5b), rearing duration ($F(4, 43) = 8.23$, $P < 0.01$; Table 4) and frequency ($F(4, 43) = 8.46$, $P < 0.01$; Table 4), and groom frequency ($F(4, 43) = 7.83$, $P < 0.01$; Table 4), on administration of D-amphetamine (0.12–1.2 mg/kg, s.c.). On administration of 4 mg/kg D-amphetamine, rapid reversal of this potentiation towards control levels was noted in these parameters.

3.7. Effect of haloperidol on D-amphetamine-induced hyperactivity

On administration of haloperidol (0.16–5 mg/kg, p.o.), a significant suppression of D-amphetamine (0.8 mg/kg, s.c.) induced potentiation of LMA duration ($F(5, 51) = 20.44$, $P < 0.01$; Fig. 5c) and frequency ($F(5, 51) = 24.10$, $P < 0.01$; Fig. 5d), rearing duration ($F(5, 51) = 25.12$, $P < 0.01$; Table 4) and frequency ($F(5, 51) = 30.17$, $P < 0.01$; Table 4) and groom frequency ($F(5, 51) = 46.82$, $P < 0.01$; Table 4) was recorded. In addition, administration of haloperidol alone (1.6–5 mg/kg, p.o.), produced a significant reduction in

Table 4
Amphetamine-induced potentiation of rearing and grooming and reversal with haloperidol

	Rearing		Grooming	
	Duration (s)	Frequency	Duration (s)	Frequency
Dose–response				
Saline	63.7±14.5	18.4±3.2	260.9±55.3	37.8±8.2
Amphetamine 0.12 mg/kg	177.2±26.3	53.9±8.2	538.7±68.7	80.5±11.0
Amphetamine 0.4 mg/kg	465.2±141.2	170.8±50.3*	620.5±96.8	116.6±10.5**
Amphetamine 1.2 mg/kg	970.4±263.0**	298.1±65.0**	523.9±171.5	95.7±14.2**
Amphetamine 4 mg/kg	968.6±203**	170.7±26.8*	731.6±246.6	63.4±10.1*
Reversal				
Vehicle+vehicle	128±60.2	36.4±17.1	137.3±35.8	31.4±10.7
Vehicle+amphetamine (0.8 mg/kg)	841.0±97.3**	264.2±28.8**	473.4±59.7**	125.3±8.9**
Haloperidol 0.16 (mg/kg)+Amph	670.1±138.3	203.1±38.5	436.8±53.9	106.2±9.5
Haloperidol 0.5 (mg/kg)+Amph	9.17±3.2^{++}	3.3±0.7^{++}	129.5±42.6^{++}	21.3±5.9^{++}
Haloperidol 1.6 (mg/kg)+Amph	2.95±0.9^{++}	1.8±0.5^{++}	121.4±41.8^{++}	14.4±3.6^{++}
Haloperidol 5 (mg/kg)+Amph	3.4±2.3^{++}	1.5±0.2^{++}	43.5±24.2^{++}	4.7±2.6^{++}
Vehicle+vehicle	46.2±14.5	14.3±2.7	226.6±74.1	21.6±4.5
Haloperidol 0.16 (mg/kg)+Amph	63.6±28.3	17.6±7.2	311.9±64.2	40.1±9.0
Haloperidol 0.5 (mg/kg)+Amph	15.7±13.0	4.5±2.5	250.9±79.9	28.8±8.6
Haloperidol 1.6 (mg/kg)+Amph	1.2±1.1	0.3±0.2^{x}	85.5±29.7	12.2±4.8
Haloperidol 5 (mg/kg)+Amph	0.3±0.3	0.1±0.1^{x}	6.9±6.0^{x}	0.7±0.5

Dose response: amphetamine given s.c. 30-min pre-test, $n=9$/group; *Reversal:* haloperidol given p.o. 1-h pre-test, amphetamine given s.c. 30-min pre-test, $n=8$–11/group. Data are expressed as mean±S.E.M. for the 1-h test period. *$P<0.05$, **$P<0.01$, significantly different from saline or vehicle/vehicle; $^{++}P<0.01$, significantly different from vehicle/amphetamine treated group; $^{x}P<0.05$, significantly different from vehicle/vehicle by one-way ANOVA and Dunnett's test.

LMA duration ($F(4, 35)=6.16$, $P<0.01$; Fig. 5c) and frequency ($F(5, 35)=5.71$, $P<0.01$; Fig. 5d), rearing frequency ($F(4, 35)=4.95$, $P<0.05$; Table 4) and groom duration ($F(4, 35)=4.68$, $P<0.05$; Table 4), relative to vehicle treated controls.

3.8. Angiotensin-II induced drinking

LABORAS™ detected a significant potentiation in both the duration ($F(1, 18)=54.1$, $P<0.01$; Fig. 6a) and frequency ($F(1, 18)=5.18$, $P<0.05$; Fig. 6b) of drinking on administration of angiotensin-II (i.c.v.).

4. Discussion

When utilising a new behavioural classification system, which can replace conventional scoring methods, it is essential to pharmacologically validate that system. In the present investigation, LABORAS™ was assessed for its ability to detect described agonist driven responses, and their reversal by selective antagonists, focussing on LMA, eating and drinking.

4.1. mCPP-induced hypoactivity and hypophagia

Data obtained using LABORAS™ confirmed that the non-selective $5HT_{2C}$ receptor agonist mCPP produces a robust hypoactive response, which is potently inhibited by the $5HT_{2C}$ receptor antagonist, SB242084 (Kennett and Curzon, 1988; Kennett, 1993; Kennett et al., 1994, 1997). The potency of SB242084 against mCPP-induced hypoactivity (ID_{50} 0.34±0.14 i.p. locomotor frequency, 0.6±0.02 i.p. mg/kg rearing frequency) as detected by LABORAS™, is comparable with the ID_{50} value obtained using standard LMA boxes (0.11±0.66 mg/kg; Kennett et al., 1997).

In addition, the activity of SB242084 on mCPP-induced hypophagia as detected by LABORAS™ is comparable with studies in which food consumption was measured by weight of food consumed (Samanin et al., 1979; Kennett et al., 1997). Both the present study and Kennett et al. (1997) obtained complete reversal of the impaired feeding response at a dose of 6 mg/kg of SB242084, with no effect of the antagonist when administered alone.

4.2. 8-OH-DPAT-induced hyperactivity

The hyperactive response to 8-OH-DPAT and blockade by the selective $5HT_{1A}$ receptor antagonist WAY100635, as detected by LABORAS, is also comparable with standard LMA box data, where significant reversal of the 8-OH-DPAT-induced potentiation of activity was noted at doses from 0.03 to 0.1 mg/kg using either methodology (Tricklebank et al., 1984; Duxon et al., 2000).

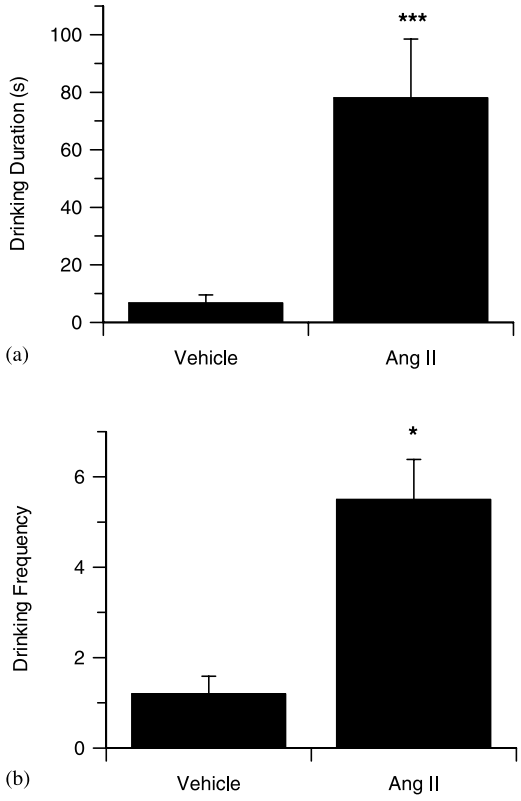


Fig. 6. (a) Angiotensin II-induced increase in drinking duration in rats. All data cited as mean ± S.E.M., $n = 10$/group. $^{**}P < 0.001$, significantly different from vehicle, by unpaired Student's t-test. (b) Angiotensin II-induced increase in drinking frequency. All data cited as mean ± S.E.M., $n = 10$/group. $^{*}P < 0.05$, significantly different from vehicle, by unpaired Student's t-test.

4.3. D-Amphetamine induced hyperactivity

In LABORAS™, D-amphetamine induced a bell-shaped dose–response relationship of LMA. At the top dose of amphetamine used in the present study (4 mg/kg), LABORAS™ detected similar levels of LMA to that which was achieved at 0.4 mg/kg. Rearing and grooming frequency were also reduced at the higher dose, which is indicative of a classical stereotypical response. This is comparable with data produced using standard LMA boxes, where doses of D-amphetamine above 2 mg/kg, were shown to produce less LMA and focussed stereotypies (Kelly et al., 1975; Costall et al., 1977; Arnt, 1995).

Reversal of D-amphetamine induced hyperactivity (0.8 mg/kg) with the classical antipsychotic haloperidol was detected by LABORAS™ in the dose range 0.5–5 mg/kg. Arnt (1995) also noted a significant inhibition of D-amphetamine-induced hyperactivity (0.5 and 2.0 mg/kg) by haloperidol, using standard LMA boxes. The reduction in home-cage activity produced by haloperidol at the doses of 1.6 and 5 mg/kg is consistent with published data, where 'acute', high dose haloperidol administration is noted to produce both hypoactivity and catalepsy in rodents (Arnt, 1995; Ushijima et al., 1995; Pillot et al., 2002).

4.4. Angiotensin II-induced drinking

LABORAS™ registered a robust increase in both drinking duration and frequency on i.c.v. administration of angiotensin II, a response commonly used for determination of correct i.c.v. cannula placement (Simpson and Routtenburg, 1973; Simpson et al., 1978). LABORAS™ is therefore capable of measuring drinking as a marker of pharmacodynamic activity.

5. Conclusions

The data described in the present study clearly show that LABORAS™ has the ability to detect 'classic' pharmacologically driven behaviours in the rat and their reversal with selective antagonists. In addition LABORAS™ has several advantages over conventional behavioural methodologies. Multiple behaviours are automatically scored in a single experiment, and the use of a home-cage environment will allow future studies of a much longer duration with continuous monitoring to be undertaken. It is clear that there is significant utility of the LABORAS™ system in the generation of high-quality pharmacodynamic data and also potentially in the temporal analysis of rodent disease modelling and, with a system adapted for mice, in the behavioural profiling of transgenic animals.

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