Psychopharmacology (2006) 184: 212–220
DOI 10.1007/s00213-005-0229-7

ORIGINAL INVESTIGATION

José Manuel Trigo · Fany Panayi · Guadalupe Soria · Rafael Maldonado · Patricia Robledo

A reliable model of intravenous MDMA self-administration in naïve mice

Received: 7 June 2005 / Accepted: 22 September 2005 / Published online: 15 December 2005

Abstract *Rationale:* MDMA is one of the most widely consumed recreational drugs in Europe. However, the mechanisms involved in the reinforcing properties of MDMA are still unclear. In this sense, the establishment of a reliable model of MDMA self-administration in mice could represent an important approach to study the neuronal substrates associated with MDMA reward by using genetically modified mice. *Objectives:* To develop a reliable model of operant intravenous MDMA self-administration in drug-naïve mice. *Materials and methods:* Mice were trained to acquire intravenous self-administration of MDMA at different doses (0, 0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) on a FR1 schedule of reinforcement for 15 consecutive days. The motivational value of different doses of MDMA (0.125, 0.25 and 0.5 mg/kg/infusion) was then tested using a progressive ratio paradigm. Finally, [^{3}H]-mazindol autoradiographic studies were carried out in order to quantitatively assess presynaptic dopamine transporter (DAT) binding sites in the striatum of mice trained to self-administer MDMA (0 and 1.0 mg/kg/infusion) during 15 days. *Results:* The latency for discrimination between the active and inactive holes, as well as the *number* of animals acquiring stability criteria, varied as a function of the dose of MDMA. The mice responding for intermediate doses (0.125, 0.25 and 0.5 mg/kg/infusion) discriminated earlier than those responding for low (0.06 mg/kg/infusion) or high (1.0 mg/kg/infusion) doses. The percentage of animals achieving stability criteria increased with days of testing and was inversely proportional to the dose of MDMA. The breaking points achieved for doses of 0.125 and 0.25 mg/kg/infusion were significantly higher than for a dose of 0.5 mg/kg/infusion. No significant DAT neurotoxicity was observed in the striatum of animals self-administering MDMA at a dose of 1 mg/kg/infusion. *Conclusions:* The present results show that MDMA can be reliably self-administered by drug-naïve mice.

Keywords DAT · Ecstasy · Mazindol binding · Operant responding · Progressive ratio · Reinforcement

J. M. Trigo · F. Panayi · G. Soria · R. Maldonado (✉) · P. Robledo
Universitat Pompeu Fabra,
Calle Dr. Aiguader, 80,
08003 Barcelona, Spain
e-mail: rafael.maldonado@upf.edu
Tel.: +34-93-5422830

P. Robledo
Institut Municipal d'Investigació Mèdica (IMIM),
Barcelona, Spain

Introduction

MDMA (3,4-methylenedioxymethamphetamine) is a ring-substituted amphetamine derivative widely used as a recreational drug. The positive acute effects of MDMA in humans include euphoria, feelings of pleasure and emotional closeness, but it can also produce negative effects such as mental confusion, behavioural hyperactivity and hyperthermia (Parrott 2001). Long-term MDMA use can induce persistent neuropsychological disorders such as depression, psychosis and cognitive impairment (for review, see Parrott 2001; Green et al. 2003). Although MDMA is mostly used occasionally in the context of rave parties with users consuming one or two tablets, some experienced individuals may consume 10 or 25 tablets in a single session. In addition, some less frequent erratic patterns of MDMA use are now being described such as "bingeing" or intravenous administration (see Parrott 2005 for review). However, there are very limited data on how these changing patterns of MDMA use can influence the development of addictive behaviour.

In experimental animals, MDMA increases locomotion and induces rewarding effects. Indeed, conditioned place preference to MDMA administration has been shown in rats (Bilsky et al. 1991) and mice (Salzmann et al. 2003; Robledo et al. 2004a,b). The mesolimbic dopamine system has been implicated in the rewarding effects of MDMA as revealed in electrophysiological and neurochemical studies (Yamamoto and Spanos 1988; Marona-Lewicka et al. 1996; White et al. 1996; Bilsky et al. 1998; Kankaanpää et

al. 1998; Robledo et al. 2004b). However, MDMA also increases the synaptic availability of serotonin (Iravani et al. 2000) by blockade of serotonin uptake sites, and the subsequent activation of different types of serotonin receptors has been shown to modulate MDMA-induced reward (Fletcher et al. 2002; Fantegrossi et al. 2002; see Cole and Sumnall 2003 for review). Using the operant intravenous self-administration technique, the reinforcing efficacy of MDMA was demonstrated in monkeys (Beardsley et al. 1986; Lamb and Griffiths 1987; Fantegrossi et al. 2002) and rats (Ratzenboeck et al. 2001; Schenk et al. 2003). Recently, it has been shown that the dopamine D1 antagonist, SCH 23390, is capable of attenuating the reinforcing properties of MDMA in rats (Daniela et al. 2004), and a role for serotonin has been reported in the modulation of MDMA self-administration in monkeys (Fantegrossi et al. 2002). Nevertheless, there is still a paucity of data evaluating how the different neurotransmitter systems influenced by MDMA administration interact to produce reinforcing effects.

In contrast, our knowledge as to the neuronal systems implicated in the reinforcing effects of other drugs of abuse such as cocaine (Rocha 2003; Hall et al. 2004; Soria et al. 2005), opioids (Maldonado 2003), nicotine (Castañé et al. 2005), alcohol (Schumann et al. 2003) and cannabinoids (Valverde et al. 2004) has greatly advanced with the introduction of genetically modified mice. With this in mind, the aim of the present study was to develop a model of operant intravenous MDMA self-administration in mice, a paradigm considered to be the most suitable model to study abuse liability in animals. For that purpose, we examined the acquisition and the maintenance of MDMA self-administration at different training doses, under a fixed-ratio 1 (FR1) schedule of reinforcement in naïve mice. Moreover, we studied the motivational properties of MDMA using the progressive ratio paradigm, where we evaluated the amount of work an animal was willing to perform for different doses of MDMA. Finally, since repeated administration of MDMA generally produces more prominent dopaminergic than serotonergic neurotoxicity (Colado et al. 2001; O'Shea et al. 2001; Itzhak et al. 2003), we assessed the possible changes in dopamine transporters (DAT) in mice self-administering MDMA (1.0 mg/kg/infusion) for 15 consecutive days using autoradiography of [^{3}H]-mazindol binding sites.

Material and methods

Animals

Experiments were performed in CD1 male mice, weighing 22–24 g upon their arrival in the laboratory. The mice were initially housed at five animals per cage in a room with controlled temperature (21±1°C) and humidity (65±10%), with a reversed 12-h/12-h light/dark cycle (lights off from 08:00 to 20:00) and with ad libitum food and water. The experiments took place during the dark phase. Behavioural tests and animal care were conducted in accordance with the standard ethical guidelines (National Institutes of Health, 1995; European Communities Directive 86/609 EEC) and approved by the local ethical committee (CEEA-IMAS-UPF).

Drugs

MDMA hydrochloride [(+/−) 3,4-methylenedioxymethamphetamine] was obtained from Lipomed, A.G. (Arlesheim, Switzerland), and dissolved in 0.9% physiological saline.

Drug self-administration

Apparatus Self-administration training and testing occurred in 16 operant chambers (Model ENV-307A-CT, Medical Associates, Georgia, VT, USA) equipped with two holes, one was selected as the active hole for delivering the reinforcer and the other as the inactive hole. Acquisition of drug self-administration was performed using a FR1 schedule of reinforcement such that one nose-poke in the active hole resulted in one MDMA infusion, while nose-poking in the inactive hole had no consequences. The chambers were housed in sound- and light-attenuated boxes equipped with fans to provide ventilation and ambient noise. A stimulus light, located above the active hole, was paired contingently with the delivery of the reinforcer.

Infusions were delivered in a volume of 23.5 µl over 2 s. MDMA was infused via a syringe that was mounted on a microinfusion pump (PHM-100A, Med-Associates, Georgia, VT, USA) and connected, via a Tygon tubing (0.96 mm o.d., Portex Fine Bore Polythene Tubing, Portex, Kent, England), to a single-channel liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA) and to the mouse intravenous catheter. The swivel was mounted on a counter-balanced arm above the operant chamber.

Surgery The mice were deeply anaesthetized with isoflurane (1.5–2.0%) and then implanted with indwelling i.v. silastic catheters in the right jugular vein, as previously described (Soria et al. 2005). Briefly, a 5-cm length of silastic tubing (0.3 mm inner diameter, 0.6 mm outer diameter) (Silastic, Dow Corning, United Kingdom) was fitted to a 22-gauge steel cannula (Semat, England) bent at a right angle and then embedded in a cement disk (Dentalon Plus, Heraeus Kulzer, Germany) with an underlying nylon mesh. The external jugular vein was isolated, and the catheter was inserted 1.3 cm into the vein and anchored with sutures. The remaining tubing ran subcutaneously to the cannula, which exited at the mid-scapular region. All incisions were sutured and coated with antibiotic ointment (Bactroban, GlaxoSmithKline, Spain). After surgery, the animals were individually housed and allowed 4 days for recovery before the behavioural testing. The patency of the catheters was evaluated periodically (once a week), and whenever drug self-administration behaviour appeared to deviate dramatically from the one previously observed, by infusing 0.1 ml of thiopental (5 mg/ml) through

the catheter. If prominent signs of anaesthesia were not apparent within 3 s of the infusion, the animal was removed from the experiment.

Drug self-administration procedure Four days after surgery, different groups of mice were trained to nose-poke under a FR1 schedule of reinforcement in order to receive different doses of MDMA (0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) or saline during 15 days. Daily self-administration started with a priming injection of the drug, lasted for 120 min, and was conducted 6 days per week. After each session, the mice were returned to their home-cages. The number of reinforcers was limited to 50 infusions per session except for doses of 0.125 and 0.06 mg/kg/infusion, where the number of reinforcers was limited to 100 infusions per session. Each infusion was followed by a 30-s timeout period during which an active nose-poke had no consequence. The criterion for stable acquisition of self-administration behaviour was to achieve all of the following conditions: (1) less than 20% deviation from the mean of the total number of reinforcers earned in three consecutive sessions (80% stability), (2) at least 65% responding on the active hole, and (3) a minimum of five reinforcers earned per session. For the progressive ratio study, other animals were operated and were first trained to nose-poke for different doses of MDMA (0.125, 0.25 and 0.5 mg/kg/infusion) under a FR1 schedule of reinforcement. When stability had been acquired, the mice were tested on a progressive ratio schedule for the dose they were trained on. In this paradigm, the requirement to earn an injection escalated according to the following series: 1-2-3-5-12-18-27-40-60-90-135-200-300-450-675-1000. The breaking point, defined as the last ratio completed before self-administration behaviour extinguished in a 2-h session, was determined for each animal once.

Autoradiography of [^{3}H]-mazindol binding

Twenty-four hours after the last self-administration session on day 15, the mice trained to self-administer MDMA (0 and 1.0 mg/kg/infusion) were sacrificed in order to quantitatively assess presynaptic dopamine transporter (DAT) binding sites. The [^{3}H]-mazindol autoradiographic studies were carried out according to the protocol used by Ryan et al. (2001) with slight modifications, as previously described (Robledo et al. 2004a). Briefly, the animals were decapitated and their brains were quickly removed and frozen by immersion in 2-methyl-butane surrounded by dry ice. All samples were stored at −80°C before use. Coronal sections at 20-μm thick were cut in a cryostat and thaw-mounted on gelatine/chrome-coated slides. The sections were then pre-incubated at 4°C for 15 min in 50 mM Tris buffer (pH 7.9), containing 300 mM NaCl and 5 mM KCl, before incubation for 60 min in the same buffer containing 300 nM desipramine (Sigma Chemical, Madrid Spain) and 4 nM [^{3}H]-mazindol (New England Nuclear, specific activity 24 Ci/mmol). Desipramine was included to block [^{3}H]-mazindol binding to noradrenaline uptake sites. The sections were then washed twice for 3 min in the Tris–HCl pre-incubation buffer and dried under a stream of cold-dry air. To determine non-specific binding, consecutive sections were incubated in the same buffer for 60 min at 4°C with the addition of 100 μM nomifensine (Sigma Chemical, Madrid Spain). Autoradiograms were generated by apposing the labelled tissues, together with autoradiographic standards ([^{3}H] micro-scales, Amersham), to tritium-sensitive film (Hyperfilm- [^{3}H], Amersham) for a period of 6 weeks. These were developed for 5 min at 20°C. The films were analysed and quantified with a computer-assisted video-densitometer (MCID, St. Catherine, Ontario Canada) using the standard curve generated from [^{3}H]-standards. Specific binding measured in the entire striatum was determined by subtracting the non-specific binding image from that of total binding.

Statistical analysis

For the MDMA self-administration acquisition data, responses on the active hole were analysed using two-way ANOVAs with DOSE as a between-subjects factor, and DAY as a within-subject factor for each block of 5-day training, followed by one-way ANOVA between doses for each day and Dunnett post-hoc for comparisons with saline. The breaking point values, the data for the dose-response curve, and the intake data were analysed using one-way ANOVA followed by Dunnett post-hoc test. Biochemical studies were analysed using one-way ANOVA. Differences were considered significant if the probability of error was less than 5%.

Results

Acquisition and maintenance of MDMA self-administration

Figure 1 shows the acquisition of MDMA self-administration at different doses for 15 consecutive days. The data were analysed in three blocks of 5 days each in order to maximize clarity. Because some mice were lost during the course of training, animals were added to each group until an adequate number was reached for each dose. Thus, Fig. 1 represents the data for all animals even if they did not complete 15 days of training.

During the first five sessions, some animals responding for MDMA at doses of 0.125, 0.25 and 0.5 mg/kg/infusion started to discriminate between the active and the inactive holes, and a higher percentage of animals reached stability criteria for doses of 0.125 and 0.25 mg/kg/infusion (0% at a dose of 1.0 mg/kg/infusion, 7.1% at a dose of 0.5 mg/kg/infusion, 20.8% at a dose of 0.25 mg/kg/infusion, 33.3% at a dose of 0.125 mg/kg/infusion, and 11.7% at a dose of 0.06 mg/kg/infusion). Two-way ANOVA revealed significant effects of DAY [$F(4,468)= 2.637$, $p<0.05$] and DOSE [$F(5,117)=4.186$, $p<0.01$], but with no interaction between these two factors. One-way ANOVA for each day revealed a significant effect of DOSE on days 1 [$F(5,122)=7.900$,

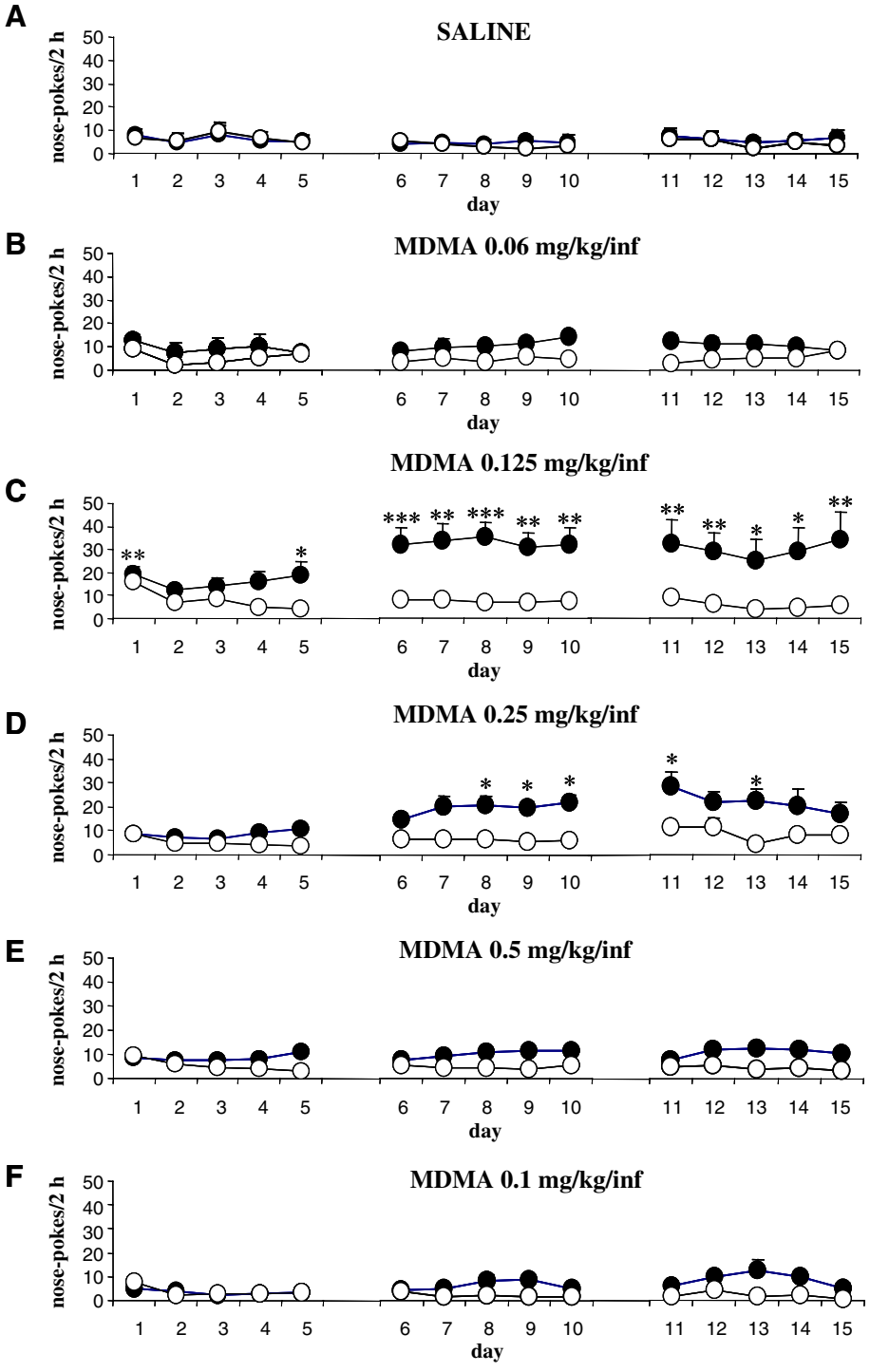

Fig. 1 Acquisition of intravenous MDMA self-administration in drug-naïve mice. Average number of nose-pokes ±SEM in both the active (*filled circles*) and the inactive (*empty circles*) holes in 2-h sessions. The 15 days of acquisition are represented in three blocks of 5 days for each of the doses tested: **a** Saline: days 1–5 (n=6), days 6–10 (n=6), and days 11–15 (n=6); **b** 0.06 mg/kg/infusion: days 1–5 (n=17), days 6–10 (n=11), and days 11–15 (n=9); **c** 0.125 mg/kg/infusion: days 1–5 (n=18), days 6–10 (n=16), and days 11–15 (n=7); **d** 0.25 mg/kg/infusion: days 1–5 (n=24), days 6–10 (n=18), and days 11–15 (n=6); **e** 0.5 mg/kg/infusion: days 1–5 (n=42), days 6–10 (n=25), and days 11–15 (n=12); and **f** 1.0 mg/kg/infusion: days 1–5 (n=16), days 6–10 (n=11), and days 11–15 (n=4). *p<0.05, **p<0.01, ***p<0.001 vs. saline for the active hole (Dunnett post-hoc test)

p<0.001] and 5 [F(5,122)= 2.901, p<0.05], with the animals responding at significantly higher rates for a dose of 0.125 mg/kg/infusion than for saline on days 1 (p<0.01) and 5 (p<0.05) (Fig. 1).

Throughout the second block of five sessions, animals responding for 0.06 and 1.0 mg/kg/infusion also started to discriminate between holes, and the percentage of animals reaching stability increased for all doses (27.2% at a dose of 1.0 mg/kg, 17.8% at a dose of 0.5 mg/kg, 52.6% at a dose of 0.25 mg/kg, 70.5% at a dose of 0.125 mg/kg and 45.4% at a dose of 0.06 mg/kg). Two-way ANOVA revealed a significant effect of DOSE [F(5,81)=8.536, p<0.001], but with no significant main effect of DAY or interaction between these two factors. One-way ANOVA for each day revealed a significant effect of DOSE on days 6 to 10 (day 6 [F(5,86)=7.633, p<0.001]; day 7 [F(5,86) =6.949, p<0.001]; day 8 [F(5,86)=7.385, p<0.001]; day 9 [F(5,86)=5.851, p<0.001]; day 10 [F(5,86)=6.065, p<0.001]),

with the animals responding at significantly higher rates for a dose of 0.125 mg/kg/infusion than for saline on days 6 to 10 ($p<0.01$), and for a dose of 0.25 mg/kg/infusion as compared to saline on days 8 to 10 ($p<0.05$) (Fig. 1).

In the last block of five sessions, the percentage of animals achieving stability criteria continued to increase: 0.06 mg/kg/infusion (66.6%), 0.125 mg/kg/infusion (80%), 0.25 mg/kg/infusion (100%), 0.5 mg/kg/infusion (47%), and 1.0 mg/kg/infusion (83.3%). Two-way ANOVA revealed a significant effect of DOSE [$F(5,38)=4.354$, $p<0.01$], but with no significant main effect of DAY or interaction between these two factors. One-way ANOVA for each day revealed a significant effect of DOSE on days 11, 12, 14 and 15 (day 11 [$F(5,43)=5.433$, $p<0.01$]; day 12 [$F(5,43)=3.264$, $p<0.05$]; day 14 [$F(5,43)=2.391$, $p<0.05$]; day 15 [$F(5,43)=3.550$, $p<0.01$]), with the animals responding at significantly higher rates for a dose of 0.125 mg/kg/infusion than for saline on days 11 to 15 ($p<0.01$ and $p<0.05$ at days 11, 12, 15 and 13, 14, respectively), and for a dose of 0.25 mg/kg/infusion as compared to saline on day 11 and 13 ($p<0.05$) (Fig. 1).

The average number of sessions required to achieve stability criteria varied as a function of the dose, such that fewer number of sessions were needed for animals acquiring MDMA self-administration at intermediate doses (Mean±SEM=5.58±0.63 and 6.27±0.71, for doses of 0.125 and 0.25 mg/kg/infusion, respectively), and more sessions were needed for animals acquiring MDMA self-administration at lower doses (Mean±SEM=7.33±1.35 at a dose of 0.06 mg/kg/infusion) and higher doses (Mean±SEM=8.0±1.38 and 9.40±0.68 at doses of 0.5 and 1.0 mg/kg/infusion, respectively).

Table 1 presents the number of infusions taken on each day by those animals that completed 15 days of self-administration. The statistical analysis performed for each block of 5 days showed similar results to those obtained for all animals shown in Fig. 1.

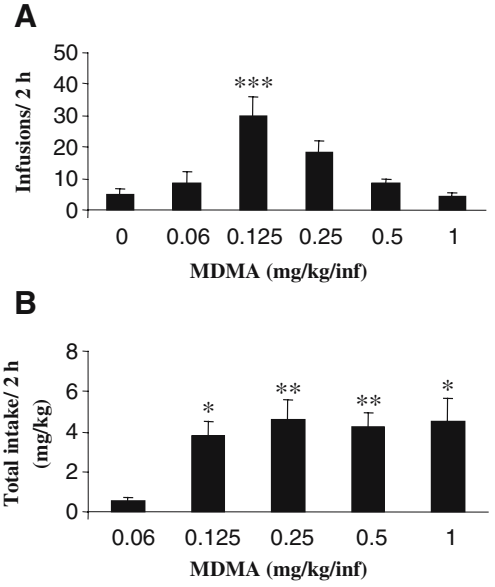

Fig. 2 The data represent the average number of infusions (**a**), and the total MDMA intake (**b**) in animals trained with different doses of MDMA (0, 0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) on day 7 of acquisition±SEM. In (**a**), ***$p<0.001$ vs. saline. In (**b**) *$p<0.05$, **$p<0.01$ vs. 0.06 mg/kg/infusion (Dunnett post-hoc test)

Figure 2a shows the dose-response curve in a 2-h session at day 7, where most doses supported discrimination between the active and the inactive holes. The total MDMA intake in a 2-h session on day 7 is shown in Fig. 2b for all the MDMA doses tested. A significant effect of dose was revealed [$F(4,80)=3.01$, $p<0.05$] and post-hoc analysis showed significant differences between doses of 0.125,

Table 1 Mean number of infusions for each day earned by the group of animals that completed the full 15 days

	Saline (N=6)	MDMA 0.06 mg/kg/infusion (N=9)	MDMA 0.125 mg/kg/infusion (N=7)	MDMA 0.25 mg/kg/infusion (N=7)	MDMA 0.5 mg/kg/infusion (N=12)	MDMA 1 mg/kg/infusion (N=4)
Day 1	7.83±2.74	12.89±3.32	18.57±4.20*	11.00±3.36	8.50±1.19	5.50±2.53
Day 2	4.50±2.17	3.78±0.86	13.14±4.17*	7.83±3.03	7.67±1.36	3.00±0.40
Day 3	8.00±3.94	3.89±1.22	15.14±4.46	9.33±4.04	7.25±1.50	1.00±0.00
Day 4	5.66±2.31	5.22±1.47	14.86±5.12	14.17±5.92	5.83±1.18	1.50±0.28
Day 5	5.66±2.60	7.00±1.72	21.00±9.57*	16.33±4.37	6.33±1.66	2.00±0.70
Day 6	4.33±1.78	8.11±2.56	28.43±9.22**	22.17±5.08*	4.92±0.60	3.00±0.57
Day 7	4.66±1.80	10.22±3.91	34.29±11.77**	26.17±6.26*	6.25±1.32	3.50±1.25
Day 8	4.00±2.44	7.89±2.48	33.86±10.19**	24.00±6.46*	7.25±1.88	7.00±3.34
Day 9	5.16±2.49	10.44±3.01	24.67±11.52*	21.50±5.10	8.58±2.54	9.25±4.25
Day 10	5.00±3.10	11.00±2.25	32.57±12.06**	26.67±6.11*	8.25±2.07	6.00±2.16
Day 11	7.50±350	11.22±2.48	29.00±8.71**	26.50±5.09*	6.83±1.75	5.75±1.37
Day 12	6.50±3.23	10.56±2.20	25.86±7.17**	20.33±3.96	11.00±2.78	9.25±1.49
Day 13	5.00±2.23	10.22±2.09	22.14±8.15*	20.83±4.25*	11.42±2.72	11.75±3.88
Day 14	5.50±2.74	9.33±2.06	25.57±9.31*	18.83±6.32	11.25±2.04	9.00±1.08
Day 15	7.00±3.62	7.89±1.25	30.29±10.52**	15.67±4.78	9.67±1.95	4.50±2.53

The number of animals in each group is shown in parenthesis
*$p<0.05$; **$p<0.01$ vs. saline (Dunnett post-hoc test)

0.25, 0.5 and 1.0 mg/kg/infusion with respect to 0.06 mg/kg/infusion ($p<0.05$, Dunnett).

Figure 3 shows representative patterns of responding by separate mice for the different doses of MDMA tested (0, 0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) in a 2-h session on day 7 of self-administration (FR1 schedule of reinforcement). The inter-response interval for MDMA self-administration showed a dose-dependent effect, being shorter for doses of 0.125 and 0.25 mg/kg/infusion than for low (0.06 mg/kg/infusion) and high (1.0 mg/kg/infusion) doses.

Progressive ratio paradigm

Figure 4 shows the breaking points achieved by mice responding on a progressive ratio schedule of reinforcement for the different doses of MDMA tested (0.125, 0.25 and 0.5 mg/kg/infusion). One-way ANOVAs showed a significant effect of the dose [$F(2,18)=8.6$, $p<0.01$], and post-hoc analysis revealed a significant difference between doses of 0.125 and 0.25 mg/kg/infusion compared to a dose of 0.5 mg/kg/infusion ($p<0.05$, Dunnett).

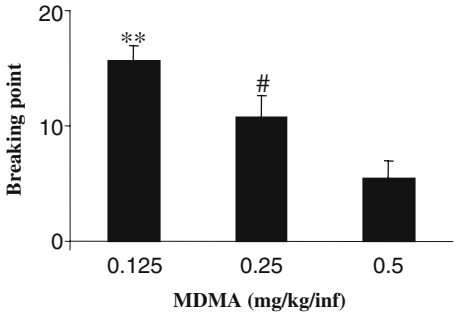

Fig. 4 MDMA self-administration under a progressive ratio schedule of reinforcement. The data represent the breaking points achieved with different doses of MDMA [0.125 ($n=5$), 0.25 ($n=6$) and 0.5 ($n=6$) mg/kg/infusion]±SEM. $^{**}p<0.01$, 0.125 mg/kg/infusion vs. 0.5 mg/kg/infusion, $^{\#}p<0.05$, 0.25 mg/kg/infusion vs. 0.5 mg/kg/infusion (Dunnett post-hoc test)

Autoradiography of [^{3}H]-mazindol binding

In order to quantitatively assess presynaptic dopamine transporter (DAT) binding sites, the mice self-administering MDMA (1.0 mg/kg/infusion) or saline during 15 days were sacrificed 1 day after the last self-administration session. The levels of [^{3}H]-mazindol binding in the striatum of animals self-administering saline (106.93± 3.18 fmol/mg, $n=6$) were similar to those obtained in previous studies in our laboratory. Thus, in two separate experiments, the animals treated with saline chronically for 4 or 6 days show levels of 80.74±7.71 and 88.5±9.7 fmol/mg, respectively. No significant differences were observed between the saline group and the group self-administering MDMA at a dose of 1.0 mg/kg/infusion (99.50±9.32 fmol/mg, $n=4$) ($p>0.05$), indicating that MDMA self-administration under these conditions does not produce neurotoxicity to DAT.

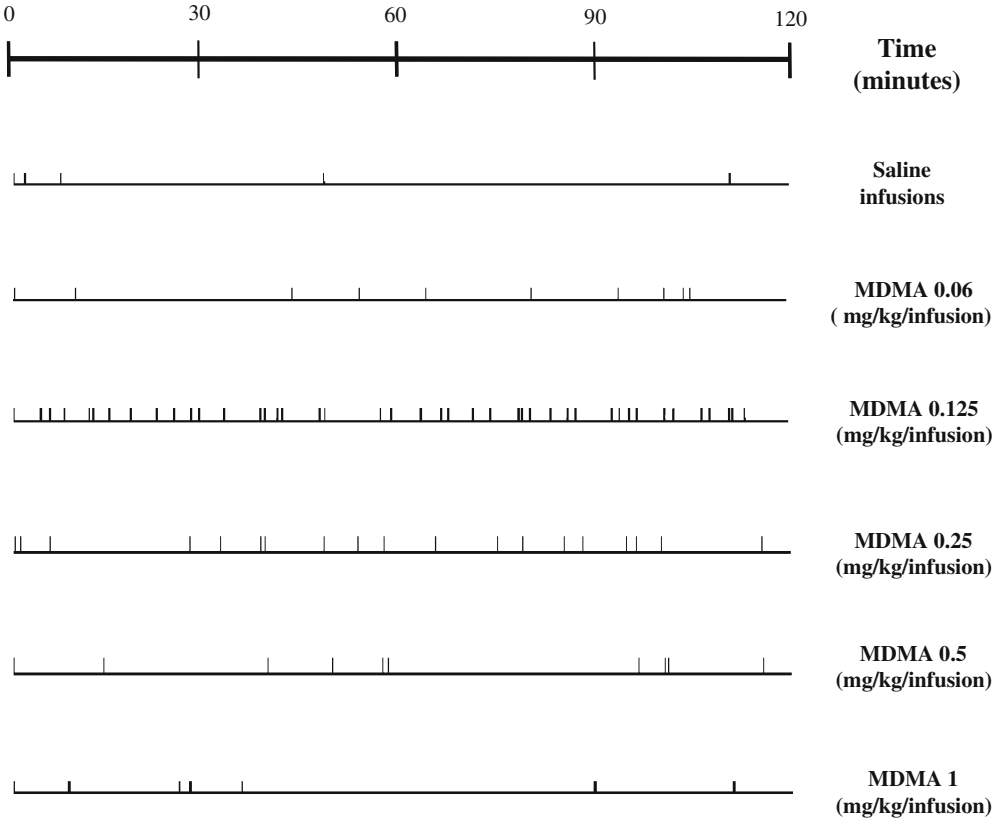

Fig. 3 Representative patterns of MDMA self-administration from animals responding for the different doses of MDMA (0, 0.06, 0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) on day 7 of training. Each *vertical line* represents one active nose-poke during a 2-h session.

Discussion

In this study, we show for the first time that it is possible to establish a reliable model of intravenous MDMA self-administration in drug-naïve mice. Dose-dependent effects were observed for acquisition of operant responding under a fixed ratio schedule of reinforcement, and for the breaking points obtained in a progressive ratio schedule.

Previous studies have shown that rats (Ratzenboeck et al. 2001; Schenk et al. 2003) and non-human primates (Beardsley et al. 1986; Lamb and Griffiths 1987; Fantegrossi et al. 2002, 2004) acquire MDMA self-administration behaviour. However, some important differences between experiments performed in monkeys and the present study in mice should be pointed out. For instance, monkeys were either trained first on cocaine and had MDMA substituted afterwards, or were maintained on cocaine while MDMA self-administration was evaluated. In contrast, our experiments were carried out in naïve mice. This is an important issue since the reinforcing properties of drugs of abuse may be affected by previous experience with other drugs (Young et al. 1981; Pierre and Vezina, 1997; Lorrain et al. 2000). However, in subsequent self-administration studies performed in rats, MDMA was self-administered by naïve as well as by cocaine-experienced rats (Ratzenboeck et al. 2001; Schenk et al. 2003). These data indicate that no previous "sensitization" of reward mechanisms is necessary for establishing MDMA self-administration in rats. An important difference we found in mice with respect to rats had to do with the temporal pattern of responding for each dose. In our experiments, we did not observe the "loading" phenomenon seen in rats (Schenk et al. 2003), rather, responses were mostly spread out during 2 h. In addition, during 15 days of MDMA self-administration, no overt changes in the pattern or the rate of responding were observed, indicating the lack of acute tolerance or sensitization to the reinforcing properties of MDMA in mice. Regarding the average drug intake per session, it is interesting to note that the mice adjusted the rate of responding for each reinforcing dose to obtain an average amount of 5 mg/kg, whereas in rats the total amount of drug intake at any given dose was four times higher (20 mg/kg) in the same time period (Schenk et al. 2003). The differences observed in the total effective amount of MDMA intake between rats and mice could be due to species differences with respect to the pharmacokinetics of MDMA (Lim et al. 1992). Interestingly, the average single-session intake of MDMA in monkeys (2 or 3 mg/kg in 1-h sessions) has been reported to be more like the one observed in mice (Fantegrossi et al. 2004).

In the current study, a dose-response curve was established for the acquisition of operant responding in different groups of untrained mice, while in previous studies with rats and monkeys, the dose-response curves were obtained once the animals had acquired self-administration of a training dose. Such an approach has the limitation of training animals with a high dose, which may influence the rate of responding for subsequent low doses. The present experimental model may be useful for future experiments intended to evaluate the neurobiological mechanism involved in the acquisition of MDMA self-administration, in particular when using genetically modified mice. Under these conditions, dose-dependent effects for the acquisition of MDMA self-administration were evidenced in several different parameters. First of all, the rate of responding observed was inversely proportional to the dose of MDMA tested. Thus, similar to what has been shown for self-administration of other psychostimulants such as cocaine, MDMA exhibited an inverted U dose-effect curve, with doses of 0.125 and 1.0 mg/kg/infusion producing the highest and lowest rate of responding, respectively. Unexpectedly, however, the number of sessions required for achieving acquisition criteria was greater in mice responding for the highest dose of 1.0 mg/kg/infusion than for those responding for intermediate doses (0.125 and 0.25 mg/kg/infusion). In addition, the percentage of mice acquiring stability was less for high than for intermediate doses of MDMA. These findings contrast with previous studies carried out in our laboratory with cocaine in mice where self-administration with high doses (1.0 mg/kg/infusion) was acquired faster and more efficiently than with low doses (0.32 mg/kg/infusion) (Soria et al. 2005). Although the specific pharmacokinetic properties of MDMA, such as its long-lasting effects, may contribute to the low rates of responding observed with high doses, it is also possible that the pharmacodynamics of MDMA may differ depending on the dose. In this regard, drug discrimination studies in rats have shown that high doses of MDMA (1.5 and 2.0 mg/kg) generalized more to the norfenfluramine-appropriate lever, whereas low doses (0.125 mg/kg) generalized more to the amphetamine-appropriate levers (Schechter 1997). These findings suggest that low doses of MDMA may produce more "dopaminergic-like" behavioural effects. In agreement, our results showing that animals acquire responding for low doses of MDMA faster and more efficiently than for higher doses support a main role of the dopaminergic system in the acquisition of MDMA self-administration. However, at present there are no clear data available in monkeys or rats to support this contention since the reinforcing potency of different doses of MDMA has not been tested in untrained animals. In addition, very few systematic studies evaluating the role of different neurotransmitter systems in the reinforcing or incentive properties of MDMA exist. One study in rats shows that the dopamine D1 antagonist, SCH 23390, produces a rightward shift in the dose-response curve for MDMA self-administration (Daniela et al. 2004). In monkeys, the effects of serotonin 5-HT_{2A} antagonists have been evaluated on the dose-response curve for each MDMA stereoisomer separately, but not for the racemic compound (Fantegrossi et al. 2002). Thus, further experiments are necessary in order to fully understand how these two neurotransmitter systems modulate the acquisition and maintenance of MDMA self-administration in experimental animals.

When the motivational strength of the different doses of MDMA was evaluated with the progressive ratio paradigm, the breaking points achieved were found to be inversely proportional to the dose of MDMA tested (0.125 mg/kg/infusion, BP=15.6±1.46; 0.25 mg/kg/infusion, BP=10.66±

2.02; and 0.5 mg/kg/infusion, BP=5.75±1.42) indicating that mice were more motivated to obtain lower than higher doses of MDMA. This was also a surprising result since the amount of work that the mice are willing to perform in the progressive ratio paradigm to seek cocaine appears to be directly proportional to the dose tested, i.e. for the highest dose tested (1 mg/kg/infusion), the mice completed approximately twice as many ratios than for the lowest dose tested (0.25 mg/kg/inf) (Colby et al. 2003). One possible explanation for these discrepancies is that cocaine and MDMA have different pharmacokinetic profiles. In this sense, both linear and non-linear kinetics have been observed for cocaine (Mets et al. 1999), while non-linear pharmacokinetics has been suggested for MDMA (de la Torre et al. 2000). Thus, it may be possible that high doses of MDMA accumulate more than high doses of cocaine, and could potentially reduce responding due to some adverse effects. Alternatively, the discrepancies observed between cocaine and MDMA may be due to differences in their pharmacodynamic profile. Thus, the behavioural effects of these two substances may diverge because of a differential activation of serotonergic, dopaminergic, and/or noradrenergic neurotransmitter systems. Interestingly, in drug discrimination studies cocaine and MDMA show asymmetric generalization (Khorana et al. 2004). These data, as well as our observations showing differences between MDMA and cocaine as to the dose-effect pattern for the speed of acquisition and for the motivation to seek the drug, may be related to differential modulation of these three neurotransmitters.

Repeated administration of MDMA has been shown to produce serotonergic neurotoxicity in several animal species (for review see Green et al. 2003). In mice, it is well established that MDMA induces more prominent dopaminergic than serotonergic neurotoxicity (Colado et al. 2001; O'Shea et al. 2001; Itzhak et al. 2003). In order to verify that prolonged exposure to self-administered MDMA did not damage dopaminergic terminals, we performed autoradiography experiments. These experiments evaluated the possible changes in [^{3}H]-mazindol binding in the striatum of mice self-administering the highest dose of MDMA tested (1.0 mg/kg) during the entire period of 15 days (average daily intake of 5 mg/kg). No neurotoxicity to DAT was evidenced in these animals as compared to those mice self-administering saline for 15 days. These findings are in line with a recent study in monkeys showing that low doses of MDMA self-administered for extended periods of time do not produce dopaminergic or serotonergic neurotoxicity (Fantegrossi et al. 2004).

In summary, the current experiments show, for the first time, that MDMA can be reliably self-administered by naïve mice. We also show that mice acquire self-administration of low doses more readily than of higher doses, and that low doses of MDMA show greater motivational strength than higher doses do. These findings provide the opportunity of using this mouse model of MDMA self-administration in future research concerning the neurobiological substrates involved in this behaviour.

Acknowledgements The authors would like to thank Dr. Fernando Berrendero for his expert help in the autoradiography experiments. This work was supported by FIS grant number 03/0305, Redes del Instituto de Salud Carlos III (C03/06 and G03/05), Marató de TV3, Generalitat de Catalunya 2002SGR00193 and European Commission grant number OJ 2003/C164-005166. FP and JMT contributed equally to this work.

References

Beardsley PM, Balster RL, Harris LS (1986) Self-administration of methylenedioxymethamphetamine (MDMA) by rhesus monkeys. Drug Alcohol Depend 18:149–157

Bilsky EJ, Hubbell CL, Delconte JD, Reid LD (1991) MDMA produces a conditioned place preference and elicits ejaculation in male rats: a modulatory role for the endogenous opioids. Pharmacol Biochem Behav 40:443–447

Bilsky EJ, Montegut MJ, Nichols ML, Reid LD (1998) CGS 10746B, a novel dopamine release inhibitor, blocks the establishment of cocaine and MDMA conditioned place preferences. Pharmacol Biochem Behav 59:215–220

Castañé A, Berrendero F, Maldonado R (2005) The role of the cannabinoid system in nicotine addiction. Pharmacol Biochem Behav (in press)

Colado MI, Camarero J, Mechan AO, Sanchez V, Esteban B, Elliott JM, Green AR (2001) A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on dopamine neurones in mouse brain. Br J Pharmacol 134:1711–1723

Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW (2003) Striatal cell type-specific overexpression of DeltaFosB enhances incentive for cocaine. J Neurosci 23:2488–2493

Cole JC, Sumnall HR (2003) The pre-clinical behavioural pharmacology of 3,4-methylenedioxymethamphetamine (MDMA). Neurosci Biobehav Rev 27:199–217

Daniela E, Brennan K, Gittings D, Hely L, Schenk S (2004) Effect of SCH 23390 on (+/−)-3,4-methylenedioxymethamphetamine hyperactivity and self-administration in rats. Pharmacol Biochem Behav 77:745–750

de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, Segura J, Cami J (2000) Non-linear pharmacokinetics of MDMA ("ecstasy") in humans. Br J Clin Pharmacol 49:104–109

Fantegrossi WE, Ullrich T, Rice KC, Woods JH, Winger G (2002) 3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") and its stereoisomers as reinforcers in rhesus monkeys: serotoninergic involvement. Psychopharmacology (Berl) 161:356–364

Fantegrossi WE, Woolverton WL, Kilbourn M, Sherman P, Yuan J, Hatzidimitriou G, Ricaurte GA, Woods JH, Winger G (2004) Behavioural and neurochemical consequences of long-term intravenous self-administration of MDMA and its enantiomers by rhesus monkeys. Neuropsychopharmacology 29:1270–1281

Fletcher PJ, Korth KM, Robinson SR, Baker GB (2002) Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement. Psychopharmacology (Berl) 62:282–291

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 55:463–508

Hall FS, Sora I, Drgonova J, Li XF, Goeb M, Uhl GR (2004) Molecular mechanisms underlying the rewarding effects of cocaine. Ann NY Acad Sci 1025:47–56

Iravani MM, Asari D, Patel J, Wieczorek WJ, Kruk ZL (2000) Direct effects of 3,4-methylenedioxymethamphetamine (MDMA) on serotonin or dopamine release and uptake in the caudate putamen, nucleus accumbens, substantia nigra reticulata, and the dorsal raphe nucleus slices. Synapse 36:275–285

Itzhak Y, Ali SF, Achat SN, Anderson KL (2003) Relevance of MDMA ("ecstasy")-induced neurotoxicity to long-lasting psychomotor stimulation in mice. Psychopharmacology (Berl) 166:241–248

Kankaanpää A, Meririnne E, Lillsunde P, Seppala T (1998) The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59:1003–1009

Khorana N, Pullagurla MR, Young R, Glennon RA (2004) Comparison of the discriminative stimulus effects of 3,4-methylenedioxymethamphetamine (MDMA) and cocaine: asymmetric generalization. Drug Alcohol Depend 74:281–287

Lamb RJ, Griffiths RR (1987) Self-injection of *d*,1-3,4-methylenedioxymethamphetamine (MDMA) in the baboon. Psychopharmacology (Berl) 91:268–272

Lim HK, Zeng S, Chei DM, Foltz RL (1992) Comparative investigation of disposition of 3,4-(methylenedioxy)methamphetamine (MDMA) in the rat and the mouse by a capillary gas chromatography–mass spectrometry assay based on perfluorotributylamine-enhanced ammonia positive ion chemical ionization. J Pharm Biomed Anal 10:657–665

Lorrain DS, Arnold GM, Vezina P (2000) Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. Behav Brain Res 107:9–19

Maldonado R (2003) Molecular biology of drug addiction. Humana, Totowa, New Jersey, p 346

Marona-Lewicka D, Rhee GS, Sprague JE, Nichols DE (1996) Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacol Biochem Behav 53:99–105

Mets B, Diaz J and Jamdar S (1999) Cocaine, norcocaine, ecgonine methylester and benzoylecgonine pharmacokinetics in the rat. Life Sci 65:1317–1328

O'Shea E, Esteban B, Camarero J, Green AR, Colado MI (2001) Effect of GBR 12909 and fluoxetine on the acute and long term changes induced by MDMA ('ecstasy') on the 5-HT and dopamine concentrations in mouse brain. Neuropharmacology 40:65–74

Parrott AC (2001) Human psychopharmacology of Ecstasy (MDMA): a review of 15 years of empirical research. Hum Psychopharmacol 16:557–577

Parrott AC (2005) Chronic tolerance to recreational MDMA (3,4-methylene-dioxymethamphetamine) or Ecstasy. J Psychopharmacol 19:71–83

Pierre PJ, Vezina P (1997) Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology (Berl) 129:277–284

Ratzenboeck E, Saria A, Kriechbaum N, Zernig G (2001) Reinforcing effects of MDMA ("ecstasy") in drug-naive and cocaine-trained rats. Pharmacology 62:138–144

Robledo P, Balerio G, Berrendero F, Maldonado R (2004a) Study of the behavioural responses related to the potential addictive properties of MDMA in mice. Naunyn Schmiedebergs Arch Pharmacol 369:338–349

Robledo P, Mendizabal V, Ortuno J, de la Torre R, Kieffer BL, Maldonado R (2004b) The rewarding properties of MDMA are preserved in mice lacking mu-opioid receptors. Eur J Neurosci 20:853–858

Rocha BA (2003) Stimulant and reinforcing effects of cocaine in monoamine transporter knockout mice. Eur J Pharmacol 479:107–115

Ryan RE, Ross SA, Drago J, Loiacono RE (2001) Dose-related neuroprotective effects of chronic nicotine in 6-hydroxydopamine treated rats, and loss of neuroprotection in α_4 nicotinic receptor subunit knockout mice. Br J Pharmacol 132:1650–1656

Salzmann J, Marie-Claire C, Le Guen S, Roques BP, Noble F (2003) Importance of ERK activation in behavioral and biochemical effects induced by MDMA in mice. Br J Pharmacol 140:831–838

Schechter MD (1997) Drug–drug discrimination: stimulus properties of drugs of abuse upon a serotonergic–dopaminergic continuum. Pharmacol Biochem Behav 56:89–96

Schenk S, Gittings D, Johnstone M, Daniela E (2003) Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rat. Psychopharmacology (Berl) 169:21–27

Schumann G, Spanagel R, Mann K (2003) Candidate genes for alcohol dependence: animal studies. Alcohol Clin Exp Res 27:880–888

Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, Maldonado R, Valverde O (2005) Lack of CB-1 cannabinoid receptor impairs cocaine self-administration. Neuropsychopharmacology 30:1670–1680

Valverde O, Robledo P, Maldonado R (2004) Involvement of the endogenous opioid system in cannabinoid-induced responses. Curr Med Chem 4:183–193

White SR, Obradovic T, Imel KM, Wheaton MJ (1996) The effects of methylenedioxymethamphetamine (MDMA, "Ecstasy") on monoaminergic neurotransmission in the central nervous system. Prog Neurobiol 49:455–479

Yamamoto BK, Spanos LJ (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. Eur J Pharmacol 148:195–203

Young AM, Herling S, Woods JH (1981) History of drug exposure as a determinant of drug self-administration. NIDA Res Monogr 37:75–88