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Pharmacology, Biochemistry and Behavior 79 (2004) 331–342
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Preexposure to MDMA ("Ecstasy") delays acquisition but facilitates MDMA-induced reinstatement of amphetamine self-administration behavior in rats

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Received 23 January 2004; received in revised form 6 August 2004; accepted 9 August 2004
Available online 13 September 2004

Abstract

The current experiment investigated the effect of 3,4-methylenedioxymethamphetamine (MDMA; 'Ecstasy') preexposure on the acquisition of intravenous amphetamine self-administration and the reinstatement of amphetamine-seeking behavior by either MDMA or amphetamine. Rats were preexposed to a 5-HT depleting regime of MDMA (5 mg/kg every hour for 4 h on two consecutive days) or equivalent vehicle injections. Intravenous self-administration of low dose D-amphetamine (0.03 mg/kg/infusion) on a FR1 schedule was subsequently assessed. The rats were then given 2 weeks of extinction and tested for drug-seeking behavior with priming doses of amphetamine or MDMA. Brains were analysed for monoamine content using high-performance liquid chromatography (HPLC). MDMA-preexposed rats were initially slower to acquire amphetamine self-administration. However, by day 6 of acquisition, there was no difference from controls. Following extinction, amphetamine (1 mg/kg, i.p.) reinstated drug seeking and produced locomotor hyperactivity in both MDMA- and vehicle-pretreated animals. However, MDMA (5 mg/kg, i.p.) was only effective in producing amphetamine seeking and hyperactivity in MDMA-pretreated rats. MDMA pretreatment caused significant decreases in 5-hydroxy-indolacetic acid (5-HIAA) and 5-HT in several brain regions. These results suggest that 5-HT depletion induced by MDMA may initially slow the acquisition of amphetamine self-administration but that MDMA preexposure may also sensitize animals to the locomotor stimulating and priming effects of MDMA on drug-seeking behavior.

Keywords: MDMA; Ecstasy; Amphetamine; Self-administration; Acquisition; Reinstatement; Maintenance; Extinction; Neurotoxicity; Serotonin

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") is an illicit recreational drug that promotes the release of serotonin (5-HT) and dopamine (DA) in many brain areas, including reward-relevant regions, such as the nucleus accumbens (White et al., 1994). Like other stimulants, MDMA produces a reinforcing effect in the conditioned place preference paradigm (Bilsky et al., 1990; Schechter, 1991), dose-dependently lowers the rewarding threshold for intracranial self-stimulation in rats (Hubner et al., 1988; Lin et al., 1997) and supports intravenous and intracranial self-administration in rodents (Braida and Sala, 2002; Cornish et al., 2003; Ratzenboek et al., 2001; Schenk et al., 2003).

Moderate to high MDMA doses can cause 5-HT neuron terminal degeneration in animals, leading to a lasting reduction of brain 5-HT content (Green et al., 2003). Human MDMA users also exhibit several markers of 5-HT depletion, such as blunted endocrine responses to D-fenfluramine challenges, decreased cerebrospinal 5-hydroxy-indolacetic acid (5-HIAA) concentrations and reduced density of brain 5-HT transporter sites (for review, see Parrott, 2001). The functional consequences of MDMA-

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doi:10.1016/j.pbb.2004.08.004

induced 5-HT depletion may include long-term cognitive deficits and a number of psychiatric sequelae, including anxiety and depression (Morgan, 2000; Parrott, 2001). Similar long-term effects have also been demonstrated in a variety of animal models (Bull et al., 2003; Byrne et al., 2000; Marston et al., 1999; McGregor et al., 2003a,b; Morley et al., 2001; Sprague et al., 2003).

The possibility that exposure to MDMA may modify the response to other drugs of abuse is also of concern, particularly given emerging patterns of heavy MDMA use in many countries. Recent U.S. and Australian epidemiological studies indicate that high school exposure to MDMA has almost quadrupled over the past decade, with adolescent use doubling since 1998 (Johnston et al., 2000; Landry, 2002). In some European countries, MDMA is the second most frequently used drug, following marijuana (Landry, 2002), and studies have suggested that MDMA use is associated with increasing use of other licit and illicit drugs (von Sydow et al., 2002; Yacoubian, 2002).

The dopamine system has been traditionally implicated in the rewarding effects of stimulants (Kelly and Iversen, 1976; Lyness et al., 1979; Taylor and Robbins, 1986), while the serotonin system has sometimes been thought to have an inhibitory effect on brain reward systems (Harrison and Markou, 2001; Kelland and Chiodo, 1996; Saito et al., 1996). However, the effects of serotonergic manipulations on stimulant self-administration are rather complex and may depend upon the 5-HT receptor subtype targeted and the aspect of self-administration behavior that is being measured. Both amphetamine and cocaine self-administration are reduced by indirect 5-HT agonists (Peltier and Schenk, 1993; Porrino et al., 1989; Richardson and Roberts, 1991; Smith et al., 1986), but more selective ligands at the 5-HT receptor subtypes may either increase or decrease stimulant self-administration (Fletcher et al., 2002b). The depletion of 5-HT caused by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) increases the breakpoint for cocaine on a progressive ratio schedule (Loh and Roberts, 1990; Roberts et al., 1994) and enhances the effect of cocaine priming on reinstatement of cocaine-seeking behavior (Tran-Nguyen et al., 2001). However, cocaine seeking during extinction is reduced by 5-HT depletion (Tran-Nguyen et al., 2001). It might then be surmised that changes to the 5-HT system following MDMA exposure will have lasting effects on the response to other stimulant drugs.

Some studies have already suggested this possibility. MDMA pretreatment augmented the locomotor stimulant properties of amphetamine (Callaway and Geyer, 1992) and cocaine (Kalivas et al., 1998). In addition, MDMA pretreatment sensitized the dopaminergic response in the nucleus accumbens to a cocaine challenge (Morgan et al., 1997). More recently, exposure to a high-dose neurotoxic regime of MDMA resulted in faster acquisition of cocaine self-administration in rats (Fletcher et al., 2001). Furthermore, in adolescent mice, chronic pretreatment of MDMA has been shown to facilitate the reinstatement of cocaine-seeking behavior, as assessed by conditioned place preference (Achat-Mendes et al., 2003).

The present study sought to further investigate these phenomena by examining the effects of MDMA pretreatment on the acquisition and extinction of low-dose D-amphetamine self-administration and the reinstatement of amphetamine-seeking behavior by priming doses of amphetamine and MDMA.

2. Methods

2.1. Animals

The subjects were male albino Wistar rats (Concord Hospital, Sydney, Australia) weighing from 425 to 554 g at the start of treatment. The rats were housed individually in plastic tubs in a temperature-controlled environment (mean of 22 °C), with food and water freely available. A 12-h reversed light cycle was in operation, with lights off at 8:30 am. All testing took place during the dark cycle. All efforts were made to minimize the number of animals used and their suffering. Ethical approval was obtained from the University of Sydney Animal Ethics Committee.

2.2. Drugs

(+/−)3,4-Methylenedioxymethamphetamine hydrochloride (MDMA) was obtained from the Australian Government Analytical Laboratories (Pymble, NSW), while D-amphetamine sulphate was obtained from Sigma (Castle Hill, NSW). Both drugs were dissolved in 0.9% saline. All doses are expressed as the weight of the salt. The amphetamine solution used for intravenous self-administration experiments was filtered before use with a Millipore syringe filter (0.22 μl).

2.3. Apparatus

2.3.1. Locomotor activity during MDMA preexposure

Locomotor activity during MDMA preexposure was measured as described previously (Morley et al., 2001), in standard operant chambers (30×50×25.5 cm) with an aluminium side and Perspex front and back wall. The floor of the chamber consisted of metal bars, and the walls of the chambers were fitted with two passive infrared detectors that were triggered by movements of the head and body of the rats, as well as gross locomotion. Activity counts were recorded by a Macintosh computer running WorkbenchMac™ data acquisition software (McGregor, 1996). The test chamber was placed inside a wooden sound-attenuating box that provided darkness and masking fan noise during testing.

2.3.2. Body temperature during MDMA preexposure

Body temperature during MDMA preexposure was measured every hour using a Braun Thermoscan Instant

Thermometer (IRT 1020). The device was inserted into the ear of the rat, and a reading was provided within 3 s (Gurtman et al., 2002; Morley et al., 2001; O'Loinsigh et al., 2001). This method provides a rapid reading of body temperature in rats, which is highly correlated with rectal temperature.

2.3.3. Intravenous amphetamine self-administration

The eight operant chambers used to assess locomotor activity during MDMA preexposure were modified for use in amphetamine self-administration. Each chamber was equipped with two 5-cm-wide retractable levers (Med Associates) on the right hand wall, situated 6 cm above the grid floor and 11 cm apart. The depression of one lever (the active lever) resulted in the intravenous infusion of a 0.05-ml amphetamine solution over 2.5 s, followed by the illumination of a cue light (situated 5 cm above the lever), indicating a 20-s time out period. During this time-out period, rats could not receive drug infusions. Depression of the inactive lever had no scheduled consequences. Locomotor activity during drug self-administration was assessed via the passive infrared detector system described above. Lever presses and locomotor activity were recorded by a Macintosh computer running WorkbenchMac™ software for data acquisition (McGregor, 1996), which also controlled drug delivery.

The drug infusion system consisted of an infusion pump (Med-PC), a 10-ml syringe and a 23-gauge cut-off needle connected to Tygon tubing. The Tygon tubing was connected to a fluid swivel assembly (Instech, PA, USA) and PE50 tubing (Plastics One, VA, USA) threaded through a spring connector (CG313, Plastics One). At 2 cm from the base of the spring connector, the spring was separated, and the tubing exited to insert into the animals intravenous catheter via a 23-gauge hypodermic tubing connector (1 cm long). The spring connector was attached to the rat's head mount (See 2.4.2 Surgery).

2.3.4. Neurochemical measurements

Brain 5-HT, 5-HIAA and dopamine were assessed using high-performance liquid chromatography (HPLC), as reported previously (Gurtman et al., 2002; McGregor et al., 2003b). Briefly, the HPLC system consisted of a Shimadzu ADVP module (Kyoto, Japan) equipped with an SIL-10 autoinjector with sample cooler and LC-10 online vacuum degassing solvent delivery unit. Chromatographic control, data collection and processing were carried out using Shimadzu Class VP data software. The mobile phase consisted of 0.1 mol/l phosphate buffer (pH 3.0), 0.74 mmol/l PIC B-8 octane sulphonic acid (Waters, Australia), sodium EDTA (0.3 mmol/l) and methanol (12% v/v). The flow rate was maintained at 1 ml/min. Dopamine, 5-HIAA, 5-HT and 5-HMeT were separated by a Merck LiChrospher 100 RP-18 reversed-phase column. Quantification was achieved via a GBC LC-1210 electrochemical detector (Melbourne, Australia) equipped with a glassy carbon working electrode set at +0.75 V. The calibration curve of each standard was obtained by the concentration versus the area ratio of the standard and internal standard.

2.4. Procedure

2.4.1. MDMA preexposure

Rats were divided into one of two groups (n=16 per group), matched for body weight. Rats in the MDMA group were given 5 mg/kg MDMA (i.p.) per hour for 4 h on each of two consecutive days (2×4×5 mg/kg). We have previously shown that this regime causes reliable 5-HT depletion (Gurtman et al., 2002; McGregor et al., 2003a). Vehicle (VEH) rats received equivalent injections of saline.

Individual rats were placed in the testing chambers for 4 h in a room where the ambient temperature was set at 26–28 °C. Every 60 min, the rats were taken out of the testing cages for measurement of body temperature and to receive their next drug injection. At the end of each 4-h session, the rats were returned to their home cages in the colony.

2.4.2. Surgery

Rats were allowed 6–7 days to recover from MDMA pretreatment before surgery began.

Rats were anaesthetised with a mixture of Ketamine (100 mg/kg, i.p.) and Xylazine (12 mg/kg, i.p.) and were implanted with an intravenous catheter into the right external jugular vein. Catheters were externalised at the back and kept in place with a polypropylene mesh assembly and sutures. Catheters were constructed from 14-cm Tygon Micro Bore tubing (ID 0.06 in., OD 0.02 in.; Small Parts, FL, USA) passed through the centre of a 1.5-cm^2 polypropylene mesh square (1000; Small Parts) attached by cranioplastic cement 2.5 cm from the distal end of the catheter. Catheters were filled with 10 IU/ml heparinized saline and closed with a 23-Ga pin. Following insertion of the intravenous catheter, head mounts for the spring connector were implanted into the rat's skull using a stereotaxic apparatus (Stoelting, IL, USA). Head mounts (CG313 bent at 100°; Plastics One) were secured in place with cranioplastic cement (Vertex, Dentimex Zeist, Holland) and four screws (Small Parts) tapped into the skull.

2.4.3. Postoperative procedures

Rats were allowed 5–7 days to recover from surgery before self-administration experiments commenced. On the surgery day, and for a further 2 days, rats were treated with an analgesic (Flunixin, 2.5 mg/kg, s.c.). Catheter patency was maintained by the daily intravenous flush of 0.2 ml of antibiotic (Cephazolin Sodium, 100g/ml) in 100 IU/ml of heparinized saline. The weight and general health of animals were monitored daily after MDMA pretreatment, postsurgery and throughout the duration of the experiment.

2.4.4. Acquisition of amphetamine self-administration

Immediately prior to self-administration sessions, each rat was placed into the chamber, and the intravenous catheter was flushed with 0.1 ml of heparinized saline (10 IU/ml) and the connector to the infusion line inserted. Once the door to the sound-attenuation chamber was closed, the session began. Each session lasted 2 h, with the drug delivered on a FR1:20-s time out schedule.

Each depression of the active lever resulted in the infusion of 0.05 ml of 0.03 mg/kg of amphetamine. At the end of each session, the infusion line was disconnected, the intravenous catheter was flushed with 0.2 ml of the antibiotic solution (see above) and the catheter was closed with the pin.

The acquisition phase continued for a total of 12 daily self-administration sessions, where the number of active lever presses, inactive lever presses, drug infusions and locomotor activity was recorded. Following the 12-day acquisition phase, each catheter was tested for patency with 0.1 ml of ketamine (10 mg/kg). Rats with nonpatent catheters were excluded from the analysis. The final number of rats analysed for the acquisition phase data set was 15 and 13 for the VEH and MDMA groups, respectively.

2.4.5. Maintenance of amphetamine self-administration

Following catheter testing, rats continued through a maintenance period of 5 days until each responded within an approximate range of 10% across days. Following this, each catheter was tested for patency with 0.1 ml of ketamine (10 mg/ml), with nonpatent catheter rats being excluded from the analysis. There were nine rats from each pretreatment group contributing to the data for the maintenance phase and for the subsequent extinction and reinstatement testing.

2.4.6. Extinction of drug-taking behavior

Rats were then subjected to extinction, where 0.9% sterile saline replaced amphetamine in the 10-ml syringe of each operant chamber. All other parameters of the experiment remained the same. Rats remained on the extinction schedule until they reached a response rate that was less than 15% of their last day of maintenance. This occurred after approximately 15 days.

2.4.7. Reinstatement of drug-seeking behavior

Following extinction, rats were given several sessions in which an injection of saline (i.p.) was given immediately prior to the animal being placed in the operant chambers under normal extinction conditions. This procedure, at first, provoked a small increase in response rate, hence, it was continued daily until the rats showed habituation to this effect (responding within 15%; range of 2–4 days).

When rats had habituated to the injection procedure, they were given an injection of MDMA (5 mg/kg, i.p.) immediately before being placed in the operant chambers, under normal extinction conditions. The number of infusions, active and inactive lever presses and locomotor activity was subsequently recorded. Once rats had rehabituated to saline injections (i.p.) in daily extinction conditions (2–4 days), they were given an injection of D-amphetamine (AMPH; 1 mg/kg, i.p.) immediately before placement into the operant chambers to test for amphetamine-induced reinstatement of lever pressing.

2.4.8. Neurochemical analysis

One week following the last reinstatement session, and approximately 9 weeks following MDMA pretreatment, the rats were decapitated using a guillotine, and their brains rapidly were removed for neurochemical analysis (n=16).

A group of noncatheterised drug-free rats (n=8) of the same strain, sex, weight and age as that of the experimental rats was also decapitated, and their brains analysed. These rats provided a control for possible effects of amphetamine self-administration on neurochemical parameters.

Four regions of interest were manually dissected out over dry ice using a method described previously (McGregor et al., 2003b). Samples from the prefrontal cortex, striatum, hippocampus and amygdala were individually placed in centrifuge tubes and were stored in a freezer at −80 °C until assayed.

Tissue samples were weighed and then homogenized with a 250-μl ice-cold solution of 0.2 M perchloric acid containing 0.1% cysteine and 200 nmol/l of internal standard 5-hydroxy-*N*-methyltryptamine (5-HMeT). The homogenate was centrifuged at 15,000×g for 10 min at 4 °C, and a 20-μl aliquot of the resulting supernatant fluid was then analysed for biogenic amines by HPLC with electrochemical detection, as described above.

2.5. Statistical analysis

Temperature and locomotor activity during the MDMA preexposure phase were analysed using a two-way repeated-measures analysis of variance (ANOVA), with drug treatment (MDMA versus VEH) and time (each of the 4 h of testing) as independent variables.

The number of drug infusions received and locomotor activity over the 12-day acquisition phase of self-administration were also analysed using a two-way repeated-measures ANOVA, with group (MDMA versus VEH) and time (days 1–12) as independent variables. Active versus inactive lever responses, over the 12 days of the acquisition phase, were analysed using three-way repeated-measures ANOVA.

The responses of each pretreatment group on the last day of the maintenance phase were analysed using a one-way ANOVA. Responses during extinction were analysed by repeated-measures ANOVA.

Reinstatement data for each prime were analysed using a two-way ANOVA, with pretreatment (MDMA versus VEH) as one factor and prime (saline versus drug) treatment as the

other factor. Significant two-way interactions were further analysed by ANOVAs of simple main effects.

Neurochemical data were analysed using separate one-way ANOVA for each monoamine (or metabolite) in each brain region, followed by a Tukey–Kramer post hoc test. For clarity of exposition, HPLC values are presented as a percentage change from the control group mean in each experiment. However, all statistics for neurochemical data were performed on untransformed data.

Some data were subjected to logarithmic transformation to reduce heterogeneity of variance. Significance was set at $P<0.05$.

3. Results

3.1. Body temperature during MDMA preexposure

Body temperatures during the 2 days of MDMA preexposure are presented in Table 1. For day 1, repeated-measures ANOVA revealed a significant group effect [$F(1,26)=17.28$; $P<0.001$], a significant effect of time [$F(3,78)=43.81$, $P<0.0001$] and a significant group×time effect interaction [$F(3,78)=31.17$, $P<0.0001$]. Similar results were obtained on day 2, with a significant effect of group [$F(1,26)=21.09$, $P<0.001$], time [$F(3,78)=63.37$, $P<0.0001$] and a group×time interaction [$F(3,78)=48.37$, $P<0.0001$]. As can be seen in Table 1, MDMA administration provoked a notable hyperthermic response, which increased across the 4 h of administration.

3.2. Locomotor activity during MDMA preexposure

Locomotor activity data during MDMA preexposure are shown in Table 1. On day 1, repeated-measures ANOVA revealed a significant group effect over the 4 h of drug administration [$F(1,26)=62.81$, $P<0.001$] and significant time [$F(3,78)=5.67$, $P<0.01$] and group×time interaction effects [$F(3,78)=3.53$. $P<0.05$]. For day 2, repeated-measures ANOVA revealed a group effect [$F(1,26)=58.84$, $P<0.001$], a significant time effect [$F(3,78)=3.49$, $P<0.05$] but not a significant hour×time interaction effect ($F<1$). As Table 1 indicates, MDMA produced hyperactivity relative to saline treatment.

3.3. Acquisition of drug self-administration

Self-administered amphetamine infusions over the 12-day acquisition phase are shown in Fig. 1a. Repeated-measures ANOVA showed that there was no significant effect of group ($F<1$) but revealed a significant effect of day [$F(11,286)=7.40$; $P<0.0001$] and a significant group×day interaction effect [$F(11,286)=2.41$; $P<0.01$]. This reflects a relatively slower acquisition of self-administration in the MDMA group relative to the VEH group.

The number of lever presses on the active versus inactive levers for each group are depicted in Fig. 1c and d. ANOVA comparing active and inactive lever presses between groups across days showed that there was no significant group effect ($F<1$), a significant lever effect [active versus inactive; $F(1,26)=87.51$; $P=<0.0001$] but no significant lever×group effect ($F<1$). There were significant day [$F(11,286)=11.03$; $P=<0.0001$] and day×group effects [$F(11,286)=2.71$; $P<0.01$] but no lever×day effect ($F<1$). However, there was a significant lever×day×group interaction effect [$F(11,286)=2.99$; $P<0.001$]. This difference in groups on active versus inactive lever pressing across days again indicates that the pattern of acquisition of amphetamine self-administration differed in the MDMA- and VEH-preexposed rats.

The mean locomotor activity counts during self-administration for each group over the 12 days of acquisition is depicted in Fig. 1b. Repeated-measures ANOVA comparing activity between groups, across days, showed there was no significant group effect ($F<1$), a significant effect of day [$F(11,286)=3.80$; $P=<0.0001$] but no significant day×group interaction ($F<1.5$). The effects of day reflected a moderate increase in locomotor activity in both groups across the first few acquisition days.

3.4. Maintenance and extinction

Responses on the active lever on the final day of the maintenance phase, as well as over the 15 days of extinction, are depicted in Fig. 2a. There was no significant difference between MDMA- or VEH-pretreated rats in responding on the final day of the maintenance phase ($F<1$).

Table 1
Mean body temperature and locomotor activity counts measured during the 4 h of drug administration over two consecutive days in the MDMA ($n=16$) and VEH ($n=16$) treatment groups

Day	Group	Body temperature (°C) (S.E.M.)					Activity (S.E.M.)
		H0	H1	H2	H3	H4	Total (H0–H4)
Day 1	MDMA	37.33 (0.14)	37.10 (0.13)	37.89 (0.13)	38.80 (0.24)	39.26 (0.10)	6056.55 (318.01)
	VEH	37.44 (0.13)	37.42 (0.15)	37.61 (0.09)	37.56 (0.09)	37.63 (0.10)	3176.28 (207.49)
Day 2	MDMA	37.4 (0.09)	37.13 (0.12)	38.05 (0.10)	38.35 (0.09)	39.04 (0.07)	5959.39 (247.20)
	VEH	37.48 (0.14)	37.33 (0.08)	37.42 (0.07)	37.43 (0.10)	37.53 (0.13)	2875.28 (290.84)

H0 refers to body temperature taken immediately before the first drug injection. H1–H4 are temperatures taken after each of the four hourly injections of MDMA or VEH.

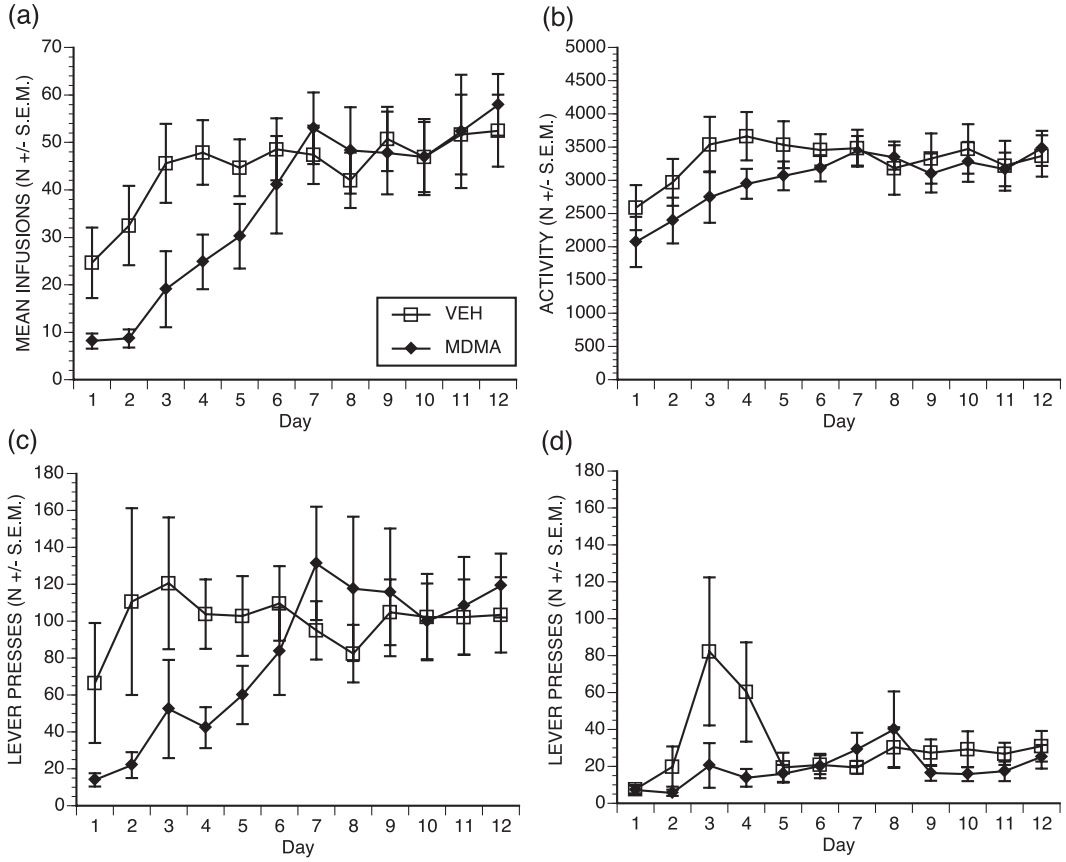

Fig. 1. The effect of MDMA preexposure on the acquisition of amphetamine self-administration. (a) Number of self-administered amphetamine infusions (0.03 mg/kg/infusion), (b) locomotor activity counts during self-administration, (c) responses on the active lever, and (d) responses on the inactive lever, over the 12 days of the acquisition phase in the VEH- and MDMA-preexposed groups. Results are expressed as mean (±S.E.M.).

Repeated-measures ANOVA revealed no significant differences between groups during extinction ($F<1$) nor any significant day×group interaction effect ($F<1$). There was a significant day effect [$F(14,224)=5.49$; $P<0.0001$], reflecting a gradual decrease in responding in both groups as the extinction phase continued.

3.5. Reinstatement

Reinstatement data are depicted in Fig. 2b–d. For the MDMA prime, the overall ANOVA on active lever presses revealed a significant main effect of prime [SALINE, MDMA; $F(1,16)=11.27$, $P<0.01$], group [VEH, MDMA; $F(1,16)=4.98$, $P<0.05$] and a prime×group interaction [$F(1,16)=4.76$, $P<0.05$]. Further analyses revealed that MDMA-pretreated rats showed significantly greater responding following the MDMA prime relative to the saline prime and also greater responding relative to the VEH group following the MDMA prime (ANOVA simple main effects, $P<0.05$).

For the AMPH prime, the overall ANOVA revealed a significant main effect of prime [SALINE, AMPH; $F(1,16)=43.23$, $P<0.0001$] but no effect of group (VEH, MDMA; $F<1.5$) and no prime×group interaction effect ($F<1$).

For responding on the inactive lever, the overall ANOVA revealed no significant main effect of prime [SALINE, MDMA; $F(1,16)<1$] and group [VEH, MDMA; $F(1,16)=3.10$, $P=0.09$] but indicated a significant prime×group interaction effect [$F(1,16)=4.43$, $P<0.05$]. However, further analyses did not reveal any significant simple main effects.

For the AMPH prime, the overall ANOVA revealed a significant main effect of prime [SALINE, AMPH; $F(1,16)=6.12$, $P<0.05$] but no effect of group [VEH, MDMA; $F<1$] or prime×group interaction ($F<1$).

For locomotor activity, the overall ANOVA revealed a significant main effect of prime [SALINE, MDMA; $F(1,16)=9.42$, $P<0.01$] and group [VEH, MDMA; $F(1,16)=12.06$, $P<0.01$] and a significant prime×group interaction effect [$F(1,16)=3.77$, $P<0.05$]. Further analyses revealed that MDMA group showed significantly greater locomotor activity following the MDMA prime relative to the saline prime and also relative to the VEH group following the MDMA prime (ANOVA simple main effects, $P<0.05$).

For the AMPH prime, the overall ANOVA revealed a significant main effect of prime [SALINE, AMPH; $F(1,16)=107.04$, $P<0.0001$] but not for group (VEH, MDMA; $F<2$) and no prime×group interaction effect ($F<1$).

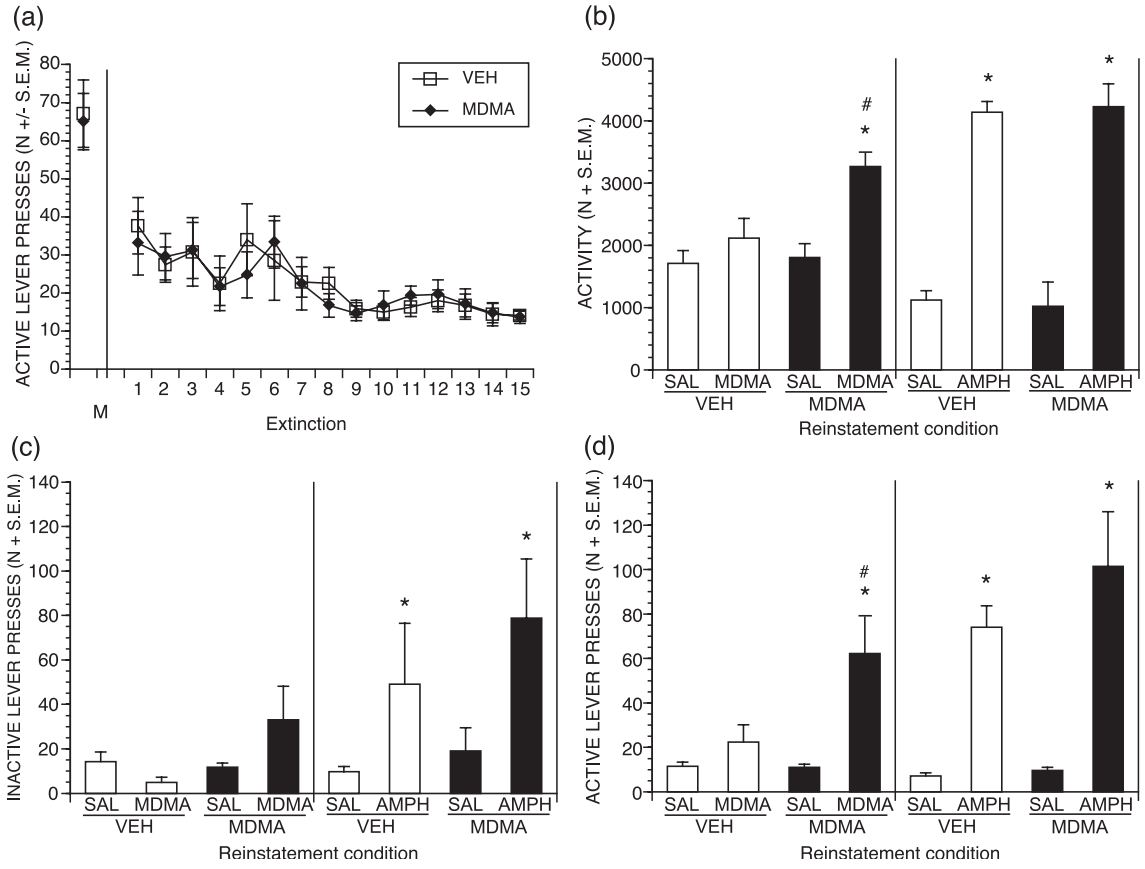

Fig. 2. The effect of MDMA preexposure on the maintenance, extinction and reinstatement of amphetamine (AMPH) seeking. (a) Responses on the active lever during the last day of the maintenance phase (M) and during extinction days 1–15 for the VEH- and MDMA-preexposed groups. (b) Locomotor activity counts. (c) Responses on the inactive lever. (d) Responses on the active lever following saline, MDMA (5 mg/kg) or AMPH (1 mg/kg) primes in the VEH- and MDMA-preexposed groups. *Significantly different to saline prime effect for same preexposure group; #significantly different to same drug prime effect in VEH-preexposed group (simple main effects ANOVA, $P<0.05$). For comparison purposes, mean responses on the active lever on the last day of the maintenance phase were 67.11 and 67.00 for VEH- and MDMA-preexposed groups, respectively. Results are expressed as mean (±S.E.M.).

3.6. Neurochemistry

The results of the HPLC analysis of neurotransmitter content in key brain regions are shown in Table 2. One-way ANOVA revealed significant differences between groups in 5-HT content in the prefrontal cortex [$F(2,21)=11.53$; $P<0.001$], striatum [$F(2,21)=4.28$; $P<0.05$], hippocampus [$F(2,21)=9.96$; $P<0.01$] and amygdala [$F(2,21)=7.13$; $P<0.05$]. ANOVA also revealed significant differences in 5-HIAA levels in the prefrontal cortex [$F(2,21)=12.45$;

Table 2
Regional changes in 5-HT, 5-HIAA and DA levels in the MDMA and CONTROL groups relative to the VEH group

Region	Treatment	5-HT	5-HIAA	DA
Prefrontal cortex	MDMA	73.36±2.83*,†	72.53±2.93*,†	122.00±12.61
	CONTROL	87.04±3.41	103.70±5.13	84.56±12.12
Striatum	MDMA	82.50±3.94*,†	79.92±3.49*,†	91.56±6.39
	CONTROL	102.10±5.14	104.37±5.35	106.44±8.06
Hippocampus	MDMA	66.66±3.66*,†	59.01±3.08*,†	81.89±15.88
	CONTROL	89.09±5.72	112.07±10.79	89.27±16.86
Amygdala	MDMA	78.64±5.12*,†	68.59±2.38*,†	79.10±10.54
	CONTROL	97.84±2.52	110.98±5.18	116.52±12.90

Data for an uncatheterised control group (CONTROL; $n=8$ per group) that did not self-administer amphetamine are also included in this analysis.
Data are presented as a percentage of the mean for VEH-preexposed rats. Absolute 5-HT values of saline-treated rats are 478.54 (22.33), 365.28 (24.53), 341.21 (19.82) and 581.97 (44.01) for the prefrontal cortex, striatum, hippocampus and amygdala, respectively, in ng/g of whole tissue. Absolute 5-HIAA values of saline-treated rats are 135.52 (6.74), 452.68.76 (30.49), 300.98 (13.73) and 313.64 (13.60) for the prefrontal cortex, striatum, hippocampus and amygdala, respectively, in ng/g of whole tissue. Absolute DA values of saline-treated rats are 615.09 (104.34), 9922.50 (560.45), 55.74 (11.79) and 303.36 (44.88) for the prefrontal cortex, striatum, hippocampus and amygdala, respectively, in ng/g of whole tissue.

* $P<0.05$ relative to VEH preexposed group, Tukey–Kramer post hoc tests.

† $P<0.05$ relative to CONTROL group, Tukey–Kramer post hoc tests.

$P<0.001$], striatum [$F(2,21)=8.74$; $P<0.01$], hippocampus [$F(2,21)=15.78$; $P<0.001$] and the amygdala [$F(2,21)=22.46$; $P<0.001$]. There were no significant group differences in dopamine in the prefrontal cortex ($F<1.55$), striatum ($F<1.5$), hippocampus ($F<1$) or the amygdala ($F<2$).

Post hoc analysis revealed that the MDMA-pretreated rats had lower 5-HT and 5HIAA levels relative to both VEH-pretreated and control rats in the prefrontal cortex, hippocampus and the amygdala. In the striatum, MDMA-treated rats had lower 5-HT and 5-HIAA levels than control rats and lower 5-HIAA levels than VEH-pretreated rats.

4. Discussion

The present results indicate that MDMA pretreatment can alter the pattern of acquisition of amphetamine self-administration, as well as increasing responsiveness to the priming and locomotor stimulant effects of MDMA.

As has been previously reported, the administration of a neurotoxic regime of MDMA resulted in marked hyperthermia and hyperactivity (Morley et al., 2001; Gurtman et al., 2002; McGregor et al., 2003b). Approximately 2 weeks later, MDMA-pretreated rats were slower to start responding to intravenous amphetamine, but their responding rapidly increased over the 12 days of the acquisition phase. In contrast, VEH-treated rats responded immediately to amphetamine and exhibited a relatively high, but flat, response curve.

A negative relationship has generally been reported between 5-HT tone and amphetamine-stimulated locomotion, conditioned reinforcement and self-administration (Fletcher, 1995; Fletcher et al., 2002a; Hollister et al., 1976). The initial attenuation of amphetamine self-administration in days 1–5 in the present study is comparatively inconsistent with these reports. However, our laboratory has observed increased 5-HT_{1B} receptor density in the nucleus accumbens accompanying MDMA-induced 5-HT depletion (McGregor et al., 2003a). This could account for the present findings, given that the activation of 5-HT_{1B} receptors in the nucleus accumbens attenuates amphetamine-conditioned responding (Fletcher et al., 2002a).

Alternatively, the slow acquisition displayed by MDMA-pretreated animals may be due to reduced exploratory behavior, increased anxiety or learning deficits, which have been reported following MDMA exposure (Byrne et al., 2000; Gurtman et al., 2002; Marston et al., 1999; McGregor et al., 2003a,b; Morley et al., 2001; Sprague et al., 2003). Previous studies indicate that animals with higher locomotor responses to a novel environment tend to acquire amphetamine self-administration, while animals with lower exploratory behavior do not (Piazza et al., 1990, 1991a,b; Pierre and Vezina, 1997). It is thus notable that rats pretreated with MDMA or methylenedioxyamphetamine (MDA) tend to display decreases in rearing, locomotion and open-field exploration (Gurtman et al., 2002; Harkin et al., 2001; McGregor et al., 2003a,b; Morley et al., 2001).

The rats employed in this study were markedly older than those used in many self-administration studies. Age has been shown to predict adverse outcome following MDMA exposure (Broening et al., 1995) and may also increase vulnerability to the potential negative effects of surgical procedures. MDMA pretreatment can significantly alter cardiovascular function (Badon et al., 2002), as well as the neurochemical response to stress (Matuszewich et al., 2002). Consequently, the exposure to surgery in older rats, combined with MDMA pretreatment, cannot be entirely discounted in hindering initial acquisition performance.

Pretreatment with MDMA, like most psychostimulants, results in enhanced conditioned place preference to cocaine (Achat-Mendes et al., 2003; Fone et al., 2002; Horan et al., 2000) and the facilitation of cocaine self-administration, particularly during the later days of acquisition (Fletcher et al., 2001). The lack of significant facilitation of amphetamine self-administration or maintenance following MDMA pretreatment in the present study contrasts with these findings. While the rewarding effects of both cocaine and amphetamine are predominantly dopaminergically mediated (Kelly and Iversen, 1976; Lyness et al., 1979; Taylor and Robbins, 1986), cocaine possesses a much higher affinity for the serotonin transporter (SERT) and causes greater serotonin release than amphetamine does (Rothman and Baumann, 2003). Thus, it is conceivable that a neurotoxic regime of MDMA may result in more marked effects on the subsequent rewarding properties of cocaine compared with amphetamine. In support of this, studies have also failed to show any marked effect of 5-HT depletion on amphetamine conditioned place preference or self-administration (Cole et al., 2003; Fletcher et al., 1999).

Furthermore, while pretreatment with a strongly 5-HT depleting dose of MDMA (20 mg/kg×2×4 days) facilitated cocaine self-administration, slower acquisition was initially observed over the first several days (Fletcher et al., 2001). In addition, preexposure to a lower dose of MDMA (5 mg/kg×1×10 days) produced a tendency towards slower acquisition of cocaine self-administration (Fletcher et al., 2001). There is a degree of similarity here with acquisition patterns reported in the current study.

The present study is the first to consider the long-term effects of MDMA on the extinction and reinstatement of self-administration behavior. MDMA pretreatment produced no clear effects on amphetamine-seeking behavior during extinction. This is consistent with the recent report that MDMA pretreatment had no effect on the extinction of cocaine conditioned place preference (Achat-Mendes et al., 2003). In contrast, the greater depletions of 5-HT produced by the tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA) or the neurotoxin 5,7-DHT attenuated

cocaine seeking during extinction of cocaine self-administration (Tran-Nguyen et al., 1999, 2001).

During reinstatement, both MDMA- and vehicle-pretreated animals demonstrated a predictable increase in drug-seeking behavior following a prime of amphetamine, an effect that has been well documented in the literature (de Wit and Stewart, 1981; Ettenberg, 1990; Gerber and Stretch, 1975; Ranaldi et al., 1999; Stretch and Gerber, 1973). However, a 5-mg/kg prime of MDMA did not produce reinstatement of amphetamine-seeking behavior in vehicle-pretreated rats.

Although MDMA and amphetamine share many similarities in their effects, the present results confirm previous work suggesting that MDMA has a unique behavioral profile relative to other psychostimulants (Fletcher et al., 2002c; Kalivas et al., 1998; Lin et al., 1997). Similarly, while two-choice drug discrimination studies have revealed inconsistent results (Baker et al., 1995; Glennon and Young, 1984; Oberlender and Nichols, 1998; Schechter, 1989), more sensitive three-choice discrimination procedures demonstrate that MDMA and amphetamine only partially substitute for each other (Baker and Taylor, 1997; Goodwin and Baker, 2000; Goodwin et al., 2003). The discriminative stimulus properties of amphetamine are clearly linked to dopaminergic systems (Schechter and Cooke, 1975), while those of MDMA may involve both dopaminergic and serotonergic mediation (Callaway et al., 1990, 1992; Gudelsky and Nash, 1996; Schechter, 1986). Specifically, low doses of MDMA may be more dopaminergic, with higher doses being more serotonergic (Schechter, 1989, 1997), although recent work suggests that 5-HT release is particularly critical to the discriminative stimulus effects of MDMA (Goodwin et al., 2003).

There was also a tendency for the MDMA-treated rats to reinstate more than the vehicle-treated rats to a 1-mg/kg amphetamine prime. Similarly, 5-HT depletion, induced by 5,7-DHT, has been shown to facilitate reinstatement of cocaine self-administration (Tran-Nguyen et al., 2001), and a recent study demonstrated that preexposure to MDMA in adolescent mice resulted in enhanced reinstatement of a conditioned place preference to cocaine (Achat-Mendes et al., 2003).

The mechanism mediating the cross-reinstatement effects produced by MDMA in MDMA-pretreated rats may involve neurochemical and behavioral sensitization. It is widely accepted that preexposure to psychostimulants induces sensitization to the effects of subsequent drug administration (Robinson and Berridge, 1993, 2000, 2001). Indeed, repeated exposure to MDMA results in a sensitization of locomotor responses (Kalivas et al., 1998; Spanos and Yamamaoto, 1989) and dopamine efflux in the nucleus accumbens to MDMA (Kalivas et al., 1998). In the current study, MDMA-pretreated rats showed significant hyperactivity to the MDMA prime, yet vehicle-pretreated animals did not. The increased responding on the active lever in MDMA-pretreated animals during MDMA-induced reinstatement was paralleled by increased responding on the inactive lever, a result that is perhaps more indicative of general behavioral sensitization than of focused reward-seeking behavior.

Interestingly, Itzhak et al. (2003) demonstrated that while brief exposure to a nonneurotoxic dose of MDMA resulted in behavioral sensitization up to 1 month afterwards, only a neurotoxic dose regimen of MDMA produced this sensitization up to 80 days later. Reinstatement in the present study was measured at periods approximately 50 days after MDMA pretreatment. Accordingly, long-lasting changes resulting from high and repeated doses of MDMA may be necessary to produce the prolonged sensitized response observed in the MDMA-pretreated animals.

It is noteworthy that the MDMA-pretreated animals displayed a tendency towards an increase in dopamine content relative to vehicle-treated animals in the prefrontal cortex and a trend towards a decrease in dopamine in the amygdala. Accordingly, it is possible that subtle dopaminergic neuroadaptations (e.g., changes in DAT and DA receptor density or affinity) may underlie some of the changes in reward-related behavior seen in the current study. However, a neurotoxic regimen of MDMA does not typically result in dopamine depletion in rats (Clemens et al., 2004; Gurtman et al., 2002; Morley et al., 2004), but consistently does so in mice (see Green et al., 2003, for review). Nonetheless, the effect of chronic amphetamine self-administration following a neurotoxic regimen of MDMA on DA or 5-HT systems is unknown and may warrant further investigation, given that an acute administration of L-DOPA or amphetamine exacerbates MDMA-induced neurotoxicity (O'Loinsigh et al., 2000; Schmidt et al., 1991). Future studies might include an additional control group that is pretreated with MDMA but does not self-administer amphetamine, to allow such an effect to be assessed.

Finally, it would clearly be of interest to also examine the effects of MDMA pretreatment on other reward-related behavior, such as food seeking. Some evidence suggests that separate neural circuits in the nucleus accumbens direct behavioral responding for cocaine compared with conventional rewards, such as food and water (Carelli et al., 2000). In this regard, future studies could usefully explore how MDMA preexposure influences the acquisition, extinction and reinstatement of operant responding for natural reinforcers.

In summary, the current findings suggest that preexposure to MDMA slows the initial acquisition of amphetamine self-administration but promotes MDMA reinstatement or amphetamine-seeking behavior and MDMA hyperactivity. Whether the latter results reflect an expression of behavioral sensitization or other long-lasting neuroadaptations resulting from serotonergic neurotoxicity remains to be elucidated. However, it seems likely that preexposure to MDMA may result in an enhanced sensitivity to psychostimulants following withdrawal, and that this is a vulnerability to relapse that may be rather long lasting.

Acknowledgements

This work was supported by an NHMRC grant to Iain S. McGregor. Kirsten Morley is the recipient of an Australian Postgraduate Award. We are grateful to Dr Jonathon Arnold for valuable comments on this manuscript.

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