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Intravenous methamphetamine self-administration in rats: Effects of intravenous or intraperitoneal MDMA co-administration

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Abstract

The combined use of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') with methamphetamine (METH) by recreational drug users is of particular concern due to their similar pharmacological and toxic profiles. In the current study we sought to elucidate why combining these particular drugs is such a popular choice among party-drug users. This was investigated through characterisation of the possible interactive effects of MDMA on METH intravenous self-administration. The first experiment involved characterisation of the METH dose–response curve for intravenous self-administration. Male Hooded–Wistar rats were trained to self-administer intravenous METH (0.01–0.3 mg/kg/infusion) and an inverted-U dose–response curve was obtained. In Experiment 2, a second squad of rats self-administered 0.01, 0.03 or 0.1 mg/kg/infusion METH and had small amounts of MDMA (0.001–0.03 mg/kg) then introduced into the infusion solution. Addition of MDMA to the METH infusion solution resulted in a dose independent reduction in responding. In Experiment 3, a third squad of rats was treated 20 min pre-session with an intraperitoneal injection of saline, 1.25 or 2.5 mg/kg of MDMA or METH to evaluate whether the reduction in responding evident in Experiment 2 was due to an MDMA-induced decrease in locomotor activity. Pre-treatment with intraperitoneal MDMA or METH had no effect on METH self-administration nor activity. We hypothesise that the reduction in METH self-administration caused by MDMA may reflect inhibitory effects of MDMA-induced 5-HT release on dopaminergic mechanisms.

Keywords: MDMA; Ecstasy; Methamphetamine; Dopamine; Serotonin; Self-administration; Reward; Reinforcement; Progressive-ratio

1. Introduction

Consuming multiple licit and illicit drugs at any one time is a regular practice among recreational drug users. While combinations such as alcohol and tobacco are particularly common, the practice of taking more than one stimulant at a time is also widely reported (Schifano et al., 1998; Degenhardt et al., 2004).

Of particular concern is the simultaneous use of the substituted amphetamines methylenedioxymethamphetamine (MDMA or 'ecstasy') and methamphetamine (METH). These drugs are often taken in combination and are both used intravenously (Boys et al., 1997; Topp et al., 1999; National Drug Strategy Household Survey, 2004; Degenhardt et al., 2005). In addition, party-drug users may inadvertently consume pills sold as 'ecstasy' that contain little pure MDMA, but rather a blend of MDMA with a cheaper additive such as METH (Australian Illicit Drug Report, 2002; Kalasinsky et al., 2004).

Combining MDMA and METH, whether intentional or not, may have serious compounding side-effects. Individually, exposure to either drug has been associated with long-term behavioural, cognitive and neurochemical deficits in rats, non-human primates and humans (Parrott, 2001; Nordahl et al., 2003; Itzhak and Achat-Mendes, 2004). This has been reflected in reports of long-term changes to the serotonergic system following MDMA, and in the dopaminergic system following METH (see Davidson et al., 2001; Green et al., 2003 for reviews).

Recent pre-clinical evidence from our laboratory suggests the combination of small amounts of METH and MDMA may lead to greater long-term neurotransmitter depletion than equivalent amounts of these drugs given alone (Clemens et al., 2004, 2005). When administered to rats, MDMA/METH combinations produced widespread depletion of both dopamine (DA) and serotonin (5-HT), and generated a unique pattern of

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noradrenaline (NA) depletion not seen with either drug alone (Clemens et al., 2005).

The motivation for party-drug users to combine MDMA with METH may reflect strong rewarding effects of this combination. As our previous research suggests a possible additive or synergistic effect of MDMA with METH on neurochemical depletion, it is possible that simultaneous use may also facilitate the individual rewarding effects MDMA or METH, thus promoting overall intake of both drugs. We sought to address this issue in the current study using an intravenous self-administration paradigm in rats.

Intravenous (IV) self-administration of METH is readily acquired and maintained in mice (Carney et al., 1991), rats (Stefanski et al., 1999; Roth and Carroll, 2004), cats (Balster et al., 1976) and non-human primates (Balster and Schuster, 1973). The powerful rewarding properties of METH in rats have also been demonstrated systemically with intraperitoneal (IP) administration in the conditioned place preference paradigm, an effect that is enhanced following pre-treatment with METH (Gehrke et al., 2003).

Similarly, IP administration of MDMA produces conditioned place preference in the rat (Meyer et al., 2002; Braida et al., 2005), and MDMA is intravenously self-administered by rats (Ratzenboeck et al., 2001; Cornish et al., 2003; Schenk et al., 2003) and non-human primates (Beardsley et al., 1986; Fantegrossi et al., 2002). MDMA pre-treatment in rats also facilitates subsequent cocaine self-administration (Fletcher et al., 2001) and MDMA-induced reinstatement to amphetamine (Morley et al., 2004). However, MDMA appears to have a relatively low reinforcing efficacy, as reflected in overall lower response rates during IV self-administration as compared to other stimulants (Lile et al., 2005). For this reason we chose to use METH as the self-administered drug upon which we could then examine the reinforcing effects of introducing MDMA.

The first aim of this study was to establish dose–response characteristics for METH IV self-administration in rats under a progressive ratio (PR) schedule of reinforcement. Previous reports describe a shallow inverted-U shaped dose–response curve, with responding maximal at 0.01–0.03 mg/kg/infusion on a fixed ratio-1 (FR-1) schedule across a 1 h session (McMillan et al., 2004), 0.03 mg/kg/infusion on a FR-3 schedule across a 2 h session (Moffett and Goeders, 2005) and slightly higher at 0.04–0.25 mg/kg/infusion across a 6-h progressive ratio schedule (Ranaldi and Poeggel, 2002; Roth and Carroll, 2004). Here we aimed to establish an IV METH self-administration dose–response curve across a 2 h session, from which we could then select appropriate doses for the second stage of this investigation.

The second aim and primary focus of this study was to explore the effect of MDMA co-administration on the rewarding efficacy of intravenous METH self-administration.

2. Methods

2.1. Subjects

The subjects were 36 male Hooded–Wistar rats purchased from the University of Adelaide or bred in our own facility at the University of Sydney. Rats weighed a mean 310±2 g at the start of the experiment and gained an average of 35.8±3.4 g in weight across the four weeks of experimentation. Rats were housed in groups of 6–8 rats per tub prior to surgery and thereafter singly housed to minimise damage to catheters. Food and water were freely available and lighting maintained on a 12 h conventional light–dark cycle (lights off at 1900 h).

All experimental procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th edition (National Health and Medical Research Council, 2004). All efforts were made to minimise the number of animals used and their suffering. Ethical approval for all experiments was obtained from the Sydney University Animal Ethics Committee.

2.2. Drugs

(+/−) 3,4-Methylenedioxymethamphetamine HCl (MDMA) and (+/−) methamphetamine HCl were purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). All drugs were dissolved in 0.9% saline. Solutions of MDMA and METH used for IV self-administration were filtered through a Millipore syringe filter (0.22 μm).

2.3. Apparatus

The self-administration apparatus and the surgical procedures were as described previously (Cornish et al., 2003, 2005; Morley et al., 2004). Eight standard operant chambers (31×50×25 cm) consisting of aluminum side panels and plexi-glass front and back panels with metal rod floor (16 rods, 6 mm diameter, 15 mm apart) were used. The chambers were housed in individual wooden sound attenuation boxes with ventilation fans providing masking noise. Miniature infrared-sensitive video cameras were mounted above each chamber and connected to television monitors positioned above the attenuation boxes, thereby allowing for continuous monitoring of each rat without interference.

Each chamber was equipped with two 5 cm-wide retractable levers (Med Associates) 6 cm above the floor and 11 cm apart. Levers were assigned as active or inactive, with the position of the active lever (left of right) counterbalanced across chambers. During the self-administration sessions, depression of the active lever resulted in a 0.05 ml infusion of METH (delivered over 3 s) and illumination of a cue light (5 cm above the lever). The cue light remained on for 20 s and represented a time-out period during which depression of the active lever had no consequence. Depression of the inactive lever had no consequences at any time.

Drug infusions were administered via infusion pumps (Med-PC, VT, USA) located external to the operant chamber but within the sound attenuation box. Activation of the pump resulted in depression of a 10 ml syringe and movement of the infusion solution through a 23-gauge cut-off needle connected to Tygon tubing. The 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, VA, USA). Approximately 20 mm from the base of the spring

connector, the spring was separated and the PE50 tubing exited to insert, via a needle-tip connector (23-gauge hypodermic tubing connector, 10 mm long), into the IV catheter, while the spring connector was attached to the head mount of the animal.

Responses on each lever and the number of drug infusions were recorded. In addition, locomotor activity was measured by two passive infrared (PIR) detectors positioned on each sidewall of the operant chamber (3 cm above floor). All data were recorded by Macintosh computers running WorkbenchMac software (McGregor, 1996).

2.4. Procedure

2.4.1. Surgery

Rats were anaesthetized with a mixture of Ketamine (100 mg/kg IP) and Xylazine (12 mg/kg IP) and implanted with an IV catheter into the right jugular vein. Catheters consisted of 140 mm of Tygon Micro Bore tubing (ID 0.02 in., OD 0.06 in., Small Parts, FL, USA) passing through the centre of a 1.5 cm^2 polypropylene mesh square (1000, Small Parts), attached by cranioplastic cement 25 mm from the distal end. The tubing of the back mount assembly was further reinforced through insertion of a 1 cm section of 23-gauge stainless steel tubing. Tubing was externalized through the back and the mesh assembly was secured in place with sutures. Catheters were filled with 10 IU/ml heparinized saline and closed with a 23-gauge pin.

Following insertion of the catheter, head mounts were affixed using a stereotaxic apparatus (Stoelting, IL, USA). Screw assemblies for spring connectors (CG313 bent at 90°; Plastics One) were embedded in cranioplastic cement (Vertex, Dentimex Zeist, Holland) and secured to the skull with four screws (Small Parts, FL, USA).

Following surgery rats were treated with an analgesic (Flunixin, 2.5 mg/kg, s.c.) and placed in cages atop warming pads, then transferred to a clean home cage upon recovery.

2.4.2. Post-operative procedures

Rats were allowed 5–7 days to fully recover from surgery before self-administration experiments commenced. For two days immediately following surgery, all rats were treated with an analgesic (Flunixin, 2.5 mg/kg/day, s.c.). Catheter patency was maintained by a daily IV flush of 0.2 ml of antibiotic (Cephazolin Sodium, 100 mg/ml) in 100 IU/ml of heparinized saline. Rat weight and general health was monitored daily post-surgery and throughout the experiment.

2.4.3. Catheter patency

At the conclusion of the self-administration acquisition phase of the experiments catheters were tested for patency with 0.1 ml of ketamine (10 mg/kg). If a catheter was no longer viable it was removed and a second catheter was inserted into the left jugular vein as described above. In this case the rat was removed from the experiment for approximately 3 days including recovery. If this second catheter lost patency the rat was excluded from the experiment.

Rats were also immediately tested for catheter patency during test phases if they failed to return to baseline responding after a drug treatment or if the number of active lever presses on a non-treatment day was ≤ 10.

2.4.4. Self-administration

All rats were trained to reliably self-administer 0.1 mg/kg/infusion of METH. At the start of each session, the rats were placed in their operant chambers and the spring connector attached to the head mount apparatus. The catheter was then flushed with 0.1 ml of heparinized saline (10 IU/ml) and the connector to the infusion line inserted and secured with a clamp. Responses on each lever, number of drug infusions delivered and locomotor activity were recorded over the 2 h self-administration session. At the conclusion of the session, the infusion line was disconnected, the catheter flushed with antibiotic (as above) and closed with a pin, and the head mount disconnected. Food and water were not available during the 2 h session and the test room was maintained at 22 °C.

Initial shaping and acquisition of lever pressing was conducted on a FR-1 schedule of reinforcement. If the rat failed to adequately self-administer METH, shaping was conducted whereby priming injections were given when the rat was in close proximity to the active lever (maximum of 5 infusions per session). During the FR acquisition phase rats were removed from the chamber following infusion of 30 or more injections to minimise potential toxic effects and avoid overdosing.

Upon satisfactory acquisition of stable self-administration (number of infusions obtained varied less than ±10% compared to previous day; approximately 6–10 days), rats were transferred to a progressive ratio (PR) schedule of reinforcement. The PR schedule of reinforcement was used here as a means of estimating the 'break point' of each rat. This represents the point at which the rat is no longer willing to press the required active lever responses to obtain an infusion, and may more accurately reflect the rewarding efficacy of the drug tested (Arnold and Roberts, 1997). It is important to note however, that with the relatively short 2 h test session used here, a true-break point, as defined by Arnold and Roberts (1997) is unlikely to be achieved. Rather, the number of self-administered infusions within the session serves as an estimate of the motivation of the rat to self-administer the drug.

The PR schedule was defined by an exponential equation in which the reinforcement number is a natural logarithmic function of the ratio value: $\text{Ratio} = ((5 \times (e^{\text{reinforcer number} \times 0.2})) - 5)$. The lever press requirement for the first 20 successive reinforcements is as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219 and 268 (Duvauchelle et al., 1998).

Each PR session lasted for 2 h and PR training sessions continued until rats had reached a stable baseline of drug infusions (number of infusions obtained varied less than ±10% compared to previous day; approximately 5 days).

2.5. Experiment 1: dose–response characteristics for METH under a progressive ratio schedule of reinforcement

Seven rats were used to establish dose–response characteristics for METH under a PR schedule of reinforcement. This was primarily used to determine a suitable self-administered dose

Table 1
Concentrations of MDMA/METH cocktails administered intravenously

	METH	MDMA
High METH	0.1	0, 0.001, 0.003, 0.01, 0.03
Moderate METH	0.03	0, 0.001, 0.003, 0.01, 0.03
Low METH	0.01	0, 0.001, 0.003, 0.01, 0.03

Note: All concentrations in final infusion solution as mg/kg/infusion.

that would be sensitive to possible modulatory effects of MDMA on METH IV self-administration in subsequent experiments.

Upon attaining a reliable baseline responding to 0.1 mg/kg/infusion of METH, the effect of altering the dose to 0.01, 0.03, 0.1 and 0.3 mg/kg/infusion of METH was examined. These doses were selected to bracket the 0.1 mg/kg/infusion dose that had been successfully used in acquisition, as well as adding an additional low dose to extend the dose–response curve. The order of dose was randomly assigned across rats. Maintenance of a stable response rate at each dose was essential before moving on to the next scheduled dose (number of infusions obtained varied less than ±10% compared to previous day; approximately 2–4 days).

2.6. Experiment 2: self-administration of MDMA and METH mixtures

Twenty-two rats were used to ascertain the effect of introducing various doses of MDMA at three levels of METH self-administration to examine whether the presence of MDMA in the infusion solution would impact on the subsequent reinforcing efficacy of the METH.

Upon attaining reliable baseline responding to 0.1 mg/kg/infusion of METH, rats were transferred either to a dose of 0.01 mg/kg/infusion, 0.03 mg/kg/infusion or remained on the initial 0.1 mg/kg/infusion. The higher 0.3 mg/kg/infusion dose used in Experiment 1 was omitted to avoid possible exacerbation of adverse behavioural effects (e.g. stereotypies, serotonin syndrome) seen at this dose (see Results section below).

Upon stabilization of responding at the new METH baseline dose, MDMA was added to the infusion solution to give a final concentration of 0, 0.001, 0.003, 0.01 or 0.03 mg/kg/infusion of MDMA in 0.01, 0.03 or 0.1 mg/kg/infusion of METH (see Table 1). The order of MDMA doses was randomly assigned across rats with each rat receiving each treatment. A change of dose occurred only after each rat reached a stable response rate at each test dose (number of infusions obtained varied less than ±10% compared to previous day).

2.7. Experiment 3: effects of pre-treatment with intraperitoneal MDMA or METH

Seven rats were used to assess the effects of pre-treatment with IP injections of MDMA or METH 20 min prior to the start of the METH self-administration session. This was to determine whether low existing levels of MDMA or METH in the rat altered the reinforcing value of, or motivation for, subsequent IV METH self-administration, and whether MDMA exposure altered the locomotor activity of rats to an extent that it may interfere with the ability of the rat to respond on the appropriate lever. Rats were trained at 0.1 mg/kg/infusion of METH as described above and thereafter maintained on a PR schedule of reinforcement. This dose was selected to be that which would be most likely to incur behavioural interference from the MDMA as a maximal total amount of drug would be administered without METH itself causing behavioural interference (e.g. stereotypical behaviours).

Prior to drug treatment all rats were habituated to the injection procedure with saline (1 ml/kg IP). A temporary increase in responding after saline injection was followed by a return to stable baseline responding within 2–6 days. Rats were then tested for the effects of pre-treatment with MDMA (1.25 and 2.5 mg/kg IP) and METH (1.25 and 2.5 mg/kg IP) on METH self-administration. These doses were selected to be high enough to be rewarding (as evidenced by conditioned place preference paradigm; (Gehrke et al., 2003; Braida et al., 2005)), yet low enough to minimise severe locomotor interference (e.g. stereotypy, head-weaving or "serotonin syndrome" behaviours) with the lever pressing behaviour (Spanos and Yamamoto, 1989; Suzuki et al., 1997; Wallace et al., 2001).

Each rat was tested (on separate days) with each pre-treatment and dose order was randomly assigned across rats. All saline and drug injections were administered 20 min prior to commencement of the self-administration session. Following each drug pre-treatment test day rats were required to return to stable baseline responding with saline pre-treatment prior to administration of the next test dose.

2.8. Statistical analysis

An initial analysis compared the responding of rats under FR versus PR schedules of reinforcement using paired samples *t*-tests. This involved comparison of active versus inactive lever pressing during baseline responding (prior to manipulation of dose, or the introduction of IP or IV MDMA).

Comparison of active versus inactive lever presses for Experiments 1 and 3 was conducted using a two-way repeated-measures analysis of variance (ANOVA) with lever (active versus inactive) and treatment (METH dose, MDMA or METH pre-treatment dose) as factors. Changes in the number of infusions received and locomotor activity counts with different drug treatments in Experiments 1 and 3 were examined using one-way repeated-measures ANOVA with response (infusions or activity) as dependent variables. Analysis of Experiment 2 was as above with an additional between subjects factor of METH dose. Randomly distributed missing values (whereby a rat completed 4 out of 5 test doses) were calculated as the median of the dose either side of that data point. Fourteen data points were calculated. Where appropriate post-hoc comparison was conducted using Fishers LSD test.

Correlation of the number of infusions received and resultant locomotor activity for each rat was assessed using a computed *Z*-score co-efficient. This was to ascertain if a relationship existed between the amount of METH infused and subsequent locomotor activity.

Table 2
Baseline responding during maintenance of methamphetamine self-administration under fixed and progressive ratio schedules of reinforcement

Lever				
Schedule	Infusions	Active	Inactive	Locomotor activity
FR-1	26.2 (2.0)	41.2 (3.5)	17.0 (4.4)	3719.2 (200.4)
PR-1	12.7 (0.5)*	302.2 (35.7)*	60.0 (15.7)*	4073.9 (191.2)

Data represents mean (SEM) for 32 rats used across Experiments 1, 2 and 3. Active indicates the total number of responses made on the active lever, including the 20-s time-out period. Locomotor activity refers to arbitrary units recorded by passive infrared detectors during the 2 h self-administration session. FR-1 data are taken as the mean of the last two days prior to transfer to the PR schedule. PR data represents the mean of the last two days prior to the start of testing (i.e. changing dose, IP MDMA or METH, or MDMA/METH combined infusion). Asterisks indicate significant difference between schedules when $p<0.05$.

All tests were performed using SPSS software (v 14.0.2, 2006). Differences were considered significant when $p<0.05$.

3. Results

3.1. Characterisation of METH self-administration under fixed ratio versus progressive ratio schedules

Table 2 reports baseline responding at the end of FR training and during subsequent PR schedules. These data are averaged across all rats used in Experiments 1, 2 and 3 ($n=32$). Paired *t*-tests revealed significantly more active than inactive lever presses on both the FR ($t(31)=5.61$; $p<0.001$) and PR ($t(31)=7.82$; $p<0.001$) schedules of reinforcement indicating successful discrimination of the drug-associated lever during pre-test training.

Responding under the PR schedule was associated with significantly more active ($t(31)=7.38$; $p<0.001$) and inactive ($t(31)=2.59$; $p<0.05$) lever presses, but with fewer total METH infusions ($t(31)=7.00$; $p<0.001$) than under the FR schedule. There was no difference in the level of locomotor activity recorded under the different schedules.

3.2. Experiment 1: dose–response characteristics for METH self-administration under a progressive ratio schedule

3.2.1. Responding across METH dose range

The effects of changing the METH infusion dose under the PR schedule are shown in Fig. 1. Repeated measures ANOVA revealed a significant effect of dose on the number of infusions administered ($F(3,18)=8.96$, $p<0.001$), the number of active lever presses ($F(3,18)=6.81$, $p<0.01$) and the amount of locomotor activity ($F(3,18)=4.82$, $p<0.05$) recorded across the 2 h test sessions.

Post-hoc analysis revealed that the 0.1 mg/kg/infusion dose was associated with significantly more infusions ($p<0.05$) and more active lever presses ($p<0.05$) than the 0.01 mg/kg/infusion dose.

3.2.2. Discrimination of the active lever at different doses

Comparison of active and inactive lever pressing across all doses revealed a significant lever effect ($F(1,12)=11.3$, $p<0.01$) indicating that the rats maintained their ability to significantly discriminate the drug-associated lever (see Fig. 1A). An overall dose effect ($F(3,12)=7.85$, $p<0.001$) demonstrated a change in responding at each dose, and a significant dose by lever interaction effect ($F(3,12)=4.73$, $p<0.01$) indicated that the change in responding across doses was specific to the active

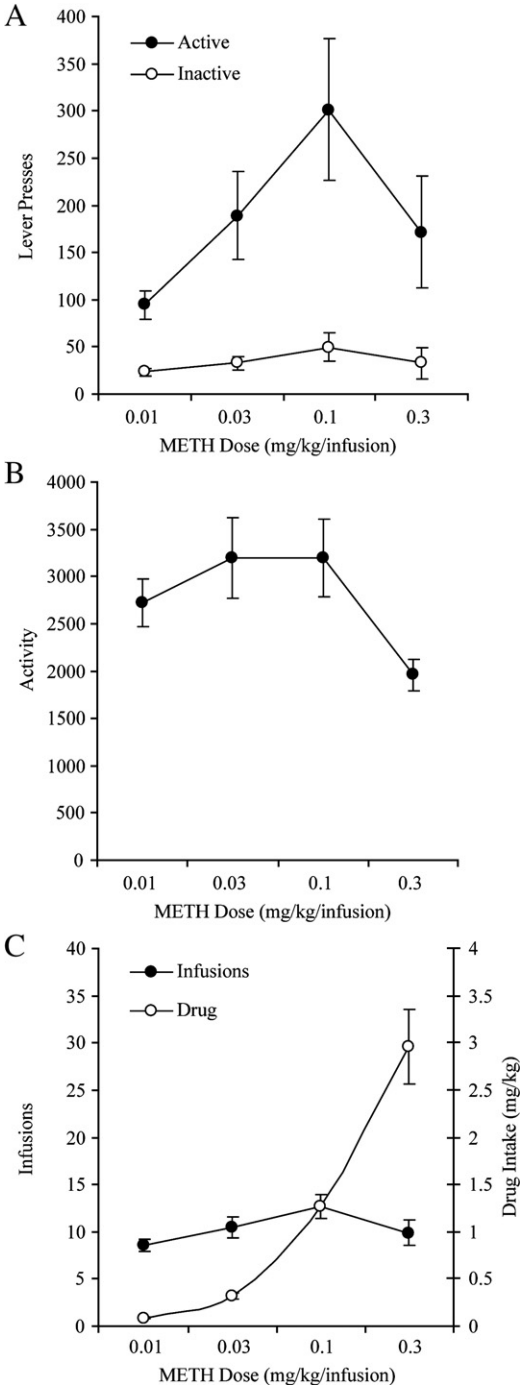

Fig. 1. Dose–response to methamphetamine intravenous self-administration across four doses under a 2-h progressive ratio schedule of reinforcement. Figures illustrate: A) number of active and inactive lever presses, B) locomotor activity (counts/min) and C) number of infusions (mg/kg/infusion) relative to total amount of methamphetamine infused (mg/kg). Points represent mean± SEM per dose of two consecutive days responding.

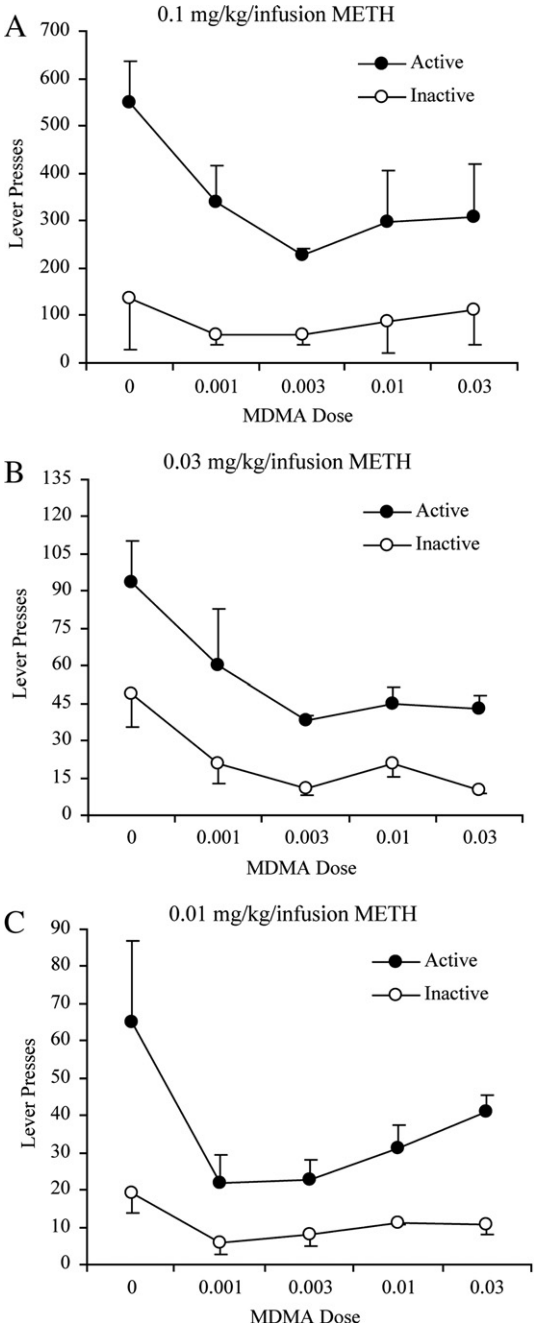

Fig. 2. The effect of introducing MDMA into the METH intravenous self-administration infusion solution on active and inactive lever pressing. Figures illustrate the effect of four doses of MDMA (final concentration in mg/kg/infusion) on responding at a final concentration of A) 0.1, B) 0.03 and C) 0.01 mg/kg/infusion of METH. Error bars represent SEM.

lever. This was supported by post-hoc analysis revealing that the number of inactive lever presses did not change with dose.

3.2.3. Locomotor activity

Locomotor activity was significantly reduced with the 0.3 mg/kg dose as compared to the 0.03 ($p<0.05$) and 0.1 ($p<0.05$) mg/kg doses (Fig. 1B). This was reflected in a significant positive correlation between infusion dose with locomotor activity with all but the highest dose (0.01 mg/kg: $Z=2.32$, $p<0.05$; 0.03 mg/kg: $Z=4.85$, $p<0.001$; 0.01 mg/kg: $Z=1.97$, $p<0.05$), indicating a dose dependent increase in locomotor activity with increasing METH infusion. A significant decrease in locomotor activity as seen at the highest dose (0.3 mg/kg) reflected the onset of stereotypical behaviour as observed by the experimenter. This was evident as repetitive head-weaving and a reduction in linear ambulatory movement.

3.2.4. Drug intake

The total amount of drug received intravenously (mg/kg) across the 2 h test session varied significantly with the dose administered ($F(3,6)=53.0$, $p<0.001$; Fig. 1C). When presented with the high dose (0.3 mg/kg/infusion) of METH, rats administered significantly greater total amount of drug than any other dose ($p<0.001$). Similarly, when rats received the 0.1 mg/kg dose, they self-administered more drug overall than either the 0.01 or 0.03 mg/kg doses ($p<0.01$). There was no significant difference in the total amount of drug administered between the 0.03 and 0.01 mg/kg/infusion doses.

3.3. Experiment 2: MDMA/METH combined IV self-administration

3.3.1. Responding across the MDMA dose range

Introduction of MDMA into the METH infusion solution resulted in an overall decrease in responding on the active lever at all METH doses (Fig. 2). Two-way repeated measures ANOVA revealed that MDMA significantly reduced the number of active lever presses achieved across the 2 h test session ($F(4,13)=10.88$, $p<0.001$), and overall post-hoc analysis revealed this to be true for all MDMA doses tested ($p<0.01$).

The reduction in active lever presses produced by MDMA co-administration occurred to a similar degree for each METH baseline dose, as evident as a non-significant interaction between changing MDMA dose with METH dose. A significant effect of METH baseline dose alone indicated that decreasing the METH dose decreased overall responding on the active lever ($F(2,16)=31.61$, $p<0.001$).

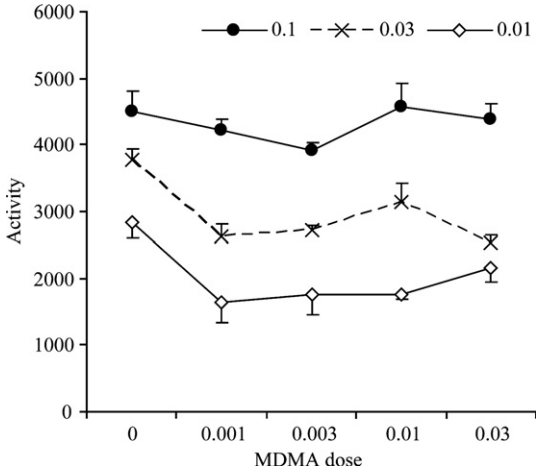

Fig. 3. The effect of introducing MDMA into the METH intravenous self-administration infusion solution on locomotor activity (counts/min). Error bars represent SEM.

Responding on the inactive lever was not affected by inclusion of MDMA into the infusion solution, nor was it significantly different for each METH baseline infusion dose.

3.3.2. Discrimination of the active versus inactive lever

Comparison of active and inactive lever pressing across all doses revealed a significant lever effect ($F(1,16)=135.40$, $p<0.001$) indicating that the rats maintained their ability to significantly discriminate the drug-associated lever at all MDMA doses (see Fig. 2). A significant MDMA dose with METH dose interaction ($F(8,28)=2.830$, $p<0.05$), but non-significant MDMA dose by METH dose by lever interaction indicates that altering MDMA dose had a differential effect on responding at each METH dose, but had no overall effect on lever discrimination.

3.3.3. Locomotor activity

Inclusion of MDMA into the infusion solution significantly reduced locomotor activity ($F(4,64)=10.03$, $p=0.001$) (Fig. 3). This reduction was similar at each METH infusion dose as indicated by non-significant interaction between MDMA dose and METH dose. An overall effect of METH dose indicates that at lower METH doses, less locomotor activity was detected ($F(2,16)=67.47$, $p<0.001$).

3.4. Experiment 3: effects of pre-treatment with intraperitoneal MDMA or METH

Intraperitoneal pre-treatment with 1.25 or 2.5 mg/kg METH or MDMA had no effect on the number of infusions, active lever presses, inactive lever presses or locomotor activity recorded during the 2 h test period (Fig. 4).

Paired *t*-tests revealed that the rats maintained the ability to significantly discriminate the active and inactive levers during METH self-administration following pre-treatment with saline ($t(2,6)=3.52$, $p<0.05$), 1.25 mg/kg METH ($t(2,6)=2.90$, $p<0.05$), 2.5 mg/kg METH ($t(2.6)=4.00$, $p<0.01$) and 2.5 mg/kg MDMA ($t(2,5)=3.04$, $p<0.05$). However, this discrimination was not evident in rats pre-treated with 1.25 mg/kg MDMA ($p<0.06$), due in the most part to one animal with a large number (170) of inactive lever responses.

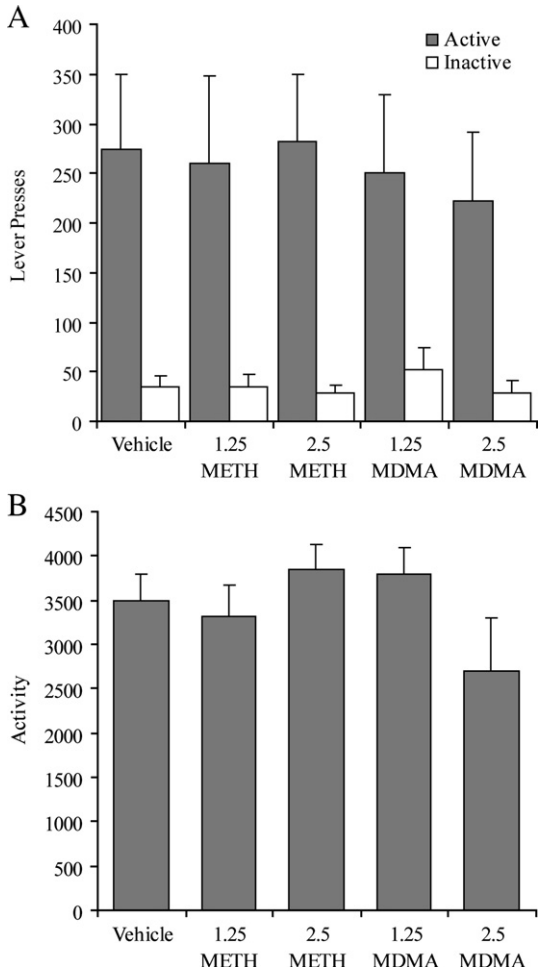

Fig. 4. The effect of intraperitoneal injection of MDMA or METH 20 min prior to the start of a 2 h 0.1 mg/kg/infusion METH self-administration session on A) the number of active versus inactive lever presses and B) locomotor activity (counts/min). Bars represent mean±SEM.

4. Discussion

The aim of this study was to characterise the effects of MDMA on IV METH self-administration. Our results indicate that inclusion of various doses of MDMA into the self-administered METH solution results in a significant decrease in IV METH self-administration. This effect was evident at three doses of METH, with responding decreasing to a similar extent at all doses. Systemic administration of MDMA had no effect on responding, nor locomotor activity, thus indicating that the decrease in responding associated with IV MDMA is due to a decrease in rewarding efficacy, rather than an overall reduction in movement.

Acquisition of IV METH self-administration occurred rapidly with rats reliably responding under both FR and PR schedules of reinforcement. Little change in inactive lever pressing across various METH infusion doses tested indicates that rats maintained the ability to successfully discriminate the active lever at all doses. Our results are in agreement with previous reports of IV METH as a potent reinforcer under both FR and PR self-administration schedules of reinforcement (Ranaldi and Poeggel, 2002; McMillan et al., 2004; Roth and Carroll, 2004; Moffett and Goeders, 2005). The dose–response curve described here was comparable to those previously reported (Ranaldi and Poeggel, 2002; McMillan et al., 2004; Roth and Carroll, 2004; Moffett and Goeders, 2005), and provided a reliable basis for dose selection in the remaining experiments.

The addition of MDMA to the METH self-administration infusion solution produced a dose independent decrease in responding on the drug-associated lever and an overall reduction in locomotor activity at three separate METH infusion doses. This result suggests that rats are sensitive to the composition of the drug being infused intravenously and are able to discriminate when MDMA has been introduced, even at very low doses.

Although rats will self-administer MDMA, the efficacy with which both acquisition and maintenance occurs is comparably low (Ratzenboeck et al., 2001; Cornish et al., 2003). Direct

comparisons of primate MDMA self-administration with other stimulants, such as cocaine, further indicate that MDMA has a low reinforcing value (Lile et al., 2005). Studies of non-amphetamine stimulants (e.g. cocaine, methylphenidate) indicate that reinforcing efficacy is strongly and positively correlated with inhibition of dopamine reuptake (Ritz et al., 1987). This is supported by evidence in monkeys and rats of amphetamine-like substances (including substituted amphetamines and phenylethylamines) that bind more potently to 5-HT transporters than DA transporters have reduced reinforcing efficacy (Munzar et al., 1999b; Wee et al., 2005).

Both MDMA and METH promote release and block reuptake of DA and 5-HT, yet with disparate potencies. As described by Rothman et al. (2001), METH more potently blocks DA reuptake (K_i=114 nM) and promotes DA release (IC_{50}=24 nM) as compared to MDMA (K_i=376 nM; IC_{50}=1572 nM). Conversely, MDMA blocks 5-HT reuptake (K_i=238 nM) and stimulates release (IC_{50}=57 nM) more effectively than METH (K_i=2,137 nM; IC_{50}=736 nM). However, studies with amphetamine-like stimulants that inhibit reuptake, but also potently release central nervous system catecholamines, indicate that reinforcing efficacy may be less related to DA reuptake inhibition but inversely correlated to 5-HT reuptake inhibition (Ritz and Kuhar, 1989). Wee et al. (2005) suggest that it is not so much the affinity for the 5-HT transporter that is important, but rather the ratio of binding to DA versus 5-HT transporters that determines reinforcing efficacy. Therefore MDMA's higher relative efficacy for 5-HT over DA may contribute to its reduced efficacy as a reinforcer relative to METH and its capacity to interfere with the usually robust reinforcing properties of METH.

The decrease in locomotor activity seen with MDMA/METH co-infusion suggests that the reduction in responding may simply be a consequence of an overall reduction in general activity. Doses in excess of those used here may produce stereotypical behaviour (e.g. head-weaving) after METH (Suzuki et al., 1997), or serotonin syndrome behaviours after MDMA (Spanos and Yamamoto, 1989). Either behaviour may interfere with the ability of the rat to maintain goal directed behaviour on the active lever. However no evidence of stereotypies or serotonin syndrome was observed in this experiment, except at the very highest METH infusion dose of 0.3 mg/kg/infusion used in Experiment 1.

Pre-treatment with IP MDMA or METH prior to the start of the METH self-administration session did not cause a detectable change in active or inactive lever presses, number of infusions received or locomotor activity under a PR reinforcement schedule. Therefore rats exposed to IP MDMA appear to be able to maintain normal locomotor behaviour, but more importantly demonstrate no MDMA-induced inhibition of the lever pressing response. The reduction of locomotor activity seen in Experiment 2 may then simply reflect a reduction in the stimulatory effects of METH as a consequence of the lesser number of METH infusions obtained. The inclusion of MDMA into the METH self-administration infusion solution therefore appears to selectively reduce the rewarding efficacy of METH in a way that is independent of locomotor activity changes.

The lack of behavioural change following IP administration of MDMA presents an interesting result in itself, suggesting exposure to MDMA, or indeed METH, through separate routes of administration has a disparate effect on the rewarding efficacy of METH when self-administered intravenously. This result is in contrast with a previous study by Munzar et al. (1999a) demonstrating a reduction in METH self-administration under a FR-5 schedule of reinforcement following pre-session IP injection of 1 mg/kg METH. The difference in schedule may be important here, as total drug intake under a 2 h PR schedule is unlikely to approach maximal METH intoxication. For example, the FR-1 schedule used in the present experiment resulted in approximately twice the number of METH infusions than that seen under the PR schedule.

The importance of schedule is further evident when comparing total drug intake per 2 h session under the FR-5 schedule adhered to by Munzar et al. (1999a) to our own under the PR schedule of reinforcement at a slightly lower dose. Under the FR-5 schedule, rats receiving a dose of 0.06 mg/kg/infusion typically received 1.22 mg/kg/2 h session. In contrast, in the current experiment rats administering a dose of 0.03 mg/kg/infusion received only 0.31 mg/kg/2 h session, and it was not until rats were administering a higher dose (more drug for less work) of 0.1 mg/kg/infusion that the amount of drug received then rose to a comparable 1.26 mg/kg/2 h session. It may then be that the additional effort required and increasing latency between infusions under a PR schedule results in a level of intoxication somewhat below that which a rat may optimally seek to obtain. Any additional drug (by IP administration in the current study) may then impact little on responding, as satiation has not occurred and motivation towards a higher level of intoxication still exists.

It might be argued that conducting tests on a PR schedule across 2 h may be insufficient to produce a true break-point, and that effects of MDMA or METH pre-treatment may have emerged had a longer test session been used. This is largely true, and the break-points reported here must be considered an estimate of that which would be achieved across a longer session length. However as MDMA exhibits a half-life of 90 min (Esteban et al., 2001), following the 20 min delay between pre-treatment and self-administration, it is likely that the maximal MDMA effects would fall well within the time of the self-administration session.

Interestingly, Munzar et al. (1999a) report a reduction in responding following pre-session treatment with the selective 5-HT releaser fenfluramine. Furthermore, when pre-session fenfluramine and the DA releaser phentermine were administered in combination, the effect was reversed, and a facilitation of METH self-administration occurred (Munzar et al., 1999a). Such a result further highlights a delicate balance between DA and 5-HT release in determining the reinforcing efficacy of METH self-administration.

The reasons for an inhibitory effect of IV but not IP treatment with MDMA are unclear. One possibility is that the doses administered IP in the current experiment are too low to elicit an effect. However slightly lower systemic doses than those administered here demonstrate positive rewarding value under the conditioned place preference paradigm for both MDMA and

METH (Gehrke et al., 2003; Braida et al., 2005). In addition, comparable pre-treatment doses of MDMA (1.25 and 2.5 mg/kg) have been shown to produce hyperactivity (Bankson and Cunningham, 2002) and robust effects on animal models of anxiety (Morley and McGregor, 2000). The doses of IP METH used (1.25 and 2.5 mg/kg) are also effective in producing hyperactivity (Szumlinski et al., 2000; Bevins and Peterson, 2004). Therefore we can be reasonably confident that the doses used here are behaviourally and pharmacologically appropriate. A different result may be achieved following repeated exposure whereby sensitisation or tolerance processes may come into effect.

Route of administration and rate of uptake may be particularly influential on subsequent reinforcing value. Volkow et al. (2003) suggest that the rewarding properties of drugs of abuse may be dependent upon the speed and magnitude of DA release, with large amounts of DA, when released quickly, contributing to greater reinforcing value. For example, the reinforcing effects of the potent DA releaser methylphenidate depend strongly on the route of administration in humans. When administered orally methylphenidate has little effect on behaviour (Volkow et al., 2001). In contrast, when administered intravenously, methylphenidate creates an intense high that positively correlates with the magnitude of dopamine release (Volkow et al., 1999). Similarly, when MDMA or METH was administered systemically in the present study, it had little effect on subsequent rewarding value of IV METH. However when MDMA is administered intravenously, it will directly interfere with the rapid efflux of DA following IV METH administration. This would produce a decrease in motivation to self-administer METH. Whether this occurs through interference with DA systems directly or as a subjective aversive effect in humans or animals (see Wee et al., 2005) remains to be investigated.

Both MDMA and METH are administered orally, nasally and intravenously by humans (Breen et al., 2003). Little literature currently exists with regard to how route of administration may affect the resultant high when drugs are co-administered. Our results suggest that co-administration of moderate MDMA and METH via non-IV routes may have little impact on the subjective or rewarding effects of IV METH. However with respect to IV METH and MDMA use, the results we report here may help to explain the low prevalence of injecting MDMA users (Breen et al., 2003), and lend support towards the targeting of the serotonin transporter in possible pharmacotherapies for IV METH abuse.

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