

Kelly J. Clemens · Petra S. van Nieuwenhuyzen ·
Kong M. Li · Jennifer L. Cornish · Glenn E. Hunt ·
Iain S. McGregor

MDMA (“ecstasy”), methamphetamine and their combination: long-term changes in social interaction and neurochemistry in the rat

Received: 30 September 2003 / Accepted: 21 December 2003 / Published online: 17 March 2004
© Springer-Verlag 2004

Abstract *Rationale:* 3,4-Methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) are illicit drugs that are increasingly used in combination. The acute and long-term effects of MDMA/METH combinations are largely uncharacterised. *Objectives:* The current study investigated the behavioural, thermal and neurotoxic effects of MDMA and METH when given alone or in combined low doses. *Methods:* Male rats received four injections, one every 2 h, of vehicle, MDMA (2.5 or 5 mg/kg per injection), METH (2.5 or 5 mg/kg per injection) or combined MDMA/METH (1.25+1.25 mg/kg per injection or 2+2 mg/kg per injection). Drugs were given at an ambient temperature of 28°C to simulate hot nightclub conditions. Body temperature, locomotor activity and head-weaving were assessed during acute drug administration while social interaction, anxiety-related behavior on the emergence test and neurochemical parameters were assessed 4–7 weeks later. *Results:* All treatments acutely increased locomotor activity, while pronounced head-weaving was seen with both MDMA/METH treatments and the higher dose METH treatment. Acute hyperthermia was greatest with the higher dose MDMA/METH treatment and was also seen with MDMA but not METH treatment. Several weeks after drug administration, both MDMA/METH groups, both METH groups and the higher dose MDMA group showed decreased social interaction relative to controls, while both MDMA/

METH groups and the lower dose MDMA group showed increased anxiety-like behaviour on the emergence test. MDMA treatment caused 5-HT and 5-HIAA depletion in several brain regions, while METH treatment reduced dopamine in the prefrontal cortex. Combined MDMA/METH treatment caused 5-HT and 5-HIAA depletion in several brain regions and a unique depletion of dopamine and DOPAC in the striatum. *Conclusions:* These results suggest that MDMA and METH in combination may have greater adverse acute effects (head-weaving, body temperature) and long-term effects (decreased social interaction, increased emergence anxiety, dopamine depletion) than equivalent doses of either drug alone.

Keywords Methamphetamine · MDMA · ecstasy · Anxiety · 5-HT · Dopamine · Polydrug use

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) is a popular illicit drug used throughout the world. Among the hazards faced by ecstasy users is that tablets sold as “ecstasy” frequently contain little or no MDMA, with other agents present either alone or in conjunction with MDMA. A common substitute found in ecstasy tablets is methamphetamine (METH, “speed”), with up to 55% of ecstasy tablets sold in Australia during 2001–2002 containing METH (Australian Bureau of Criminal Intelligence 2002). Furthermore, METH is sometimes deliberately used in combination with MDMA (Boys et al. 1997; Topp et al. 1999). The deliberate or inadvertent use of MDMA with other drugs such as METH may expose users to unpredictable adverse effects.

Numerous studies with laboratory animals suggest that MDMA and METH have long-lasting neurotoxic effects on serotonin and dopamine systems, respectively (Sabol et al. 1996; Friedman et al. 1998; Hatzidimitriou et al. 1999; Davidson et al. 2001; Gurtman et al. 2002; Green et al. 2003; McGregor et al. 2003a). MDMA acts acutely to stimulate serotonin release and block reuptake, and in

K. J. Clemens · P. S. van Nieuwenhuyzen · J. L. Cornish ·
I. S. McGregor (✉)
School of Psychology, University of Sydney,
2006 Sydney, NSW, Australia
e-mail: iain@psych.usyd.edu.au
Tel.: +61-2-93513571
Fax: +61-2-93518023

K. M. Li
Department of Pharmacology, University of Sydney,
2006 Sydney, NSW, Australia

G. E. Hunt
Department of Psychological Medicine, University of Sydney,
Concord Hospital,
2139 Sydney, NSW, Australia

sufficient doses produces a long-term depletion of brain serotonin (for review, see Green et al. 2003). Conversely, METH primarily acts acutely to block dopamine reuptake and facilitate dopamine release, while in the long term it causes a primary depletion of dopamine and, to a lesser extent, serotonin (Fleckenstein et al. 2000; Haughey et al. 2000; Kita et al. 2003).

Results from human studies indicate similar effects, with MDMA users exhibiting decreased serotonin transporter density (Buchert et al. 2003; Thomasius et al. 2003), altered 5-HT_{2A} receptor density (Reneman et al. 2002), and decreased 5-HT and 5-HIAA in cerebrospinal fluid (McCann et al. 1999; Stuerenburg et al. 2002). On the other hand, METH users show signs of long-term dopamine neuronal damage (Ernst et al. 2000) and reduced dopamine transporter density (McCann et al. 1998; Volkow et al. 2001).

Past research on the effects of combined MDMA and METH administration is scarce. METH users seeking treatment who also use MDMA showed greater drug-related problems and were less likely to complete treatment (Brecht and von Mayrhauser 2002). MDMA polydrug users demonstrated specific learning and memory deficits compared to non-MDMA polydrug users (Fox et al. 2002). Reneman and colleagues (2002) demonstrated decreased striatal dopamine transporter density in combined MDMA/METH users relative to users of MDMA only. Such studies suggest that combined MDMA/METH use may have more severe adverse effects than use of either drug alone.

Therefore the present study investigated the behavioural and neurochemical effects of combined MDMA/METH administration in the rat. The acute hyperthermic and hyperactive effects of each drug and their combination were examined as well as long-term effects on social interaction, anxiety-like behaviour on the emergence test and neurotransmitter levels. Our group has documented long-term increases in anxiety in rats given MDMA (McGregor et al. 2003a, 2003b) and here we investigated whether METH and MDMA/METH combinations have the same effect. Also of interest was whether the dopamine and serotonin depletion commonly seen with METH and MDMA respectively would be exacerbated by exposure to the drug combination.

Materials and methods

Subjects

The subjects were 63 experimentally naive male albino Wistar rats bred in our own facility, weighing an average of 376 g (± 8 g) at the start of testing. Rats were housed in groups of six to eight rats per tub, with water and food freely available. The colony room was maintained at 22°C on a 12-h reverse light cycle, with all experimentation carried out during the dark period. Rats were allocated to one of seven groups based on body weight. Different treatment conditions were equally represented within home cages. All experimentation was approved by the University of Sydney Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

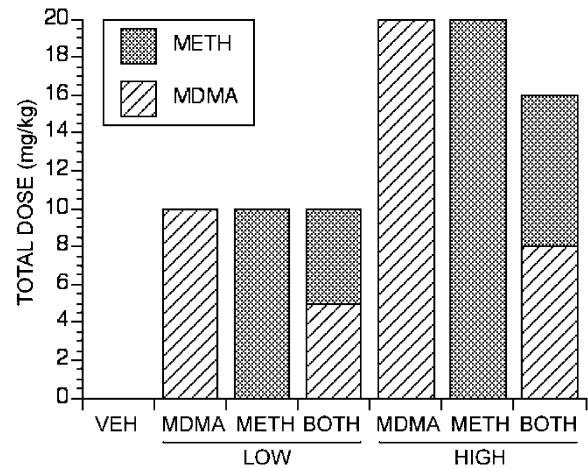


Fig. 1 Drug administration protocol. Bars represent composition and total doses of MDMA, METH and MDMA/METH combination (BOTH) administered as four injections, one every 2 h on a single day

Drug administration

(±)3,4-Methylenedioxymethamphetamine HCl and methamphetamine HCl were purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). Drugs were dissolved in 0.9% saline and injected at 1 ml/kg IP. Drugs were administered on a single day with all rats receiving four injections: one injection every 2 h. The doses used for each treatment condition are shown in Fig. 1.

Doses were selected with regard to previous studies demonstrating robust 5-HT depleting effects of MDMA given at 4×5 mg/kg, one injection every hour for 4 h, on 2 consecutive days (Gurtman et al. 2002; McGregor et al. 2003a, 2003b) and dopamine depleting effects of METH at 4×10 mg/kg, one injection every 2 h on a single day (Wallace et al. 1999; Chapman et al. 2001; Davidson et al. 2001; Bisagno et al. 2002). We selected two METH and two MDMA doses that were approximately one-half and one-quarter of these established neurotoxic doses (2.5 and 5 mg/kg MDMA or METH given every 2 h on 1 day). The approach was adopted to better model typical human doses and to allow sensitivity to possibly increased toxic effects of the MDMA/METH combinations.

The lower MDMA/METH combination dose used was 1.25 +1.25 mg/kg to give a total dose of 2.5 mg/kg per injection, matched with the total dose in the low METH and low MDMA groups. The higher dose MDMA/METH treatment involved 2+2 mg/kg per injection, giving 4 mg/kg per injection, slightly less than the 5 mg/kg per injection used in the higher dose MDMA and METH groups. This slightly lower dose was used because initial pilot studies indicated that rats given a 2.5+2.5 mg/kg MDMA/METH combination dose displayed a severe adverse reaction characterised by potentially lethal hyperthermia (>40°C).

Experimental techniques

Temperature, activity and head-weaving measurement

During acute drug treatment, rats were placed in open rectangular chambers (60×26×36 cm) with black Perspex walls and a black metal grid floor. Each rat was monitored by an infrared sensitive camera positioned above each chamber. These cameras were connected to a PC running LabView 6.1 software with a Raptor image acquisition card. A custom program written in the LabView programming environment processed the video images to detect movement of the rats within the cage during testing. Movement was measured as the number of pixels changing from black (the colour

of the background) to white (the colour of the rat) for each second of testing. This system is sensitive to extremely small movements of the rats.

The test room was maintained under low light conditions, at a high ambient temperature of $28 \pm 1^\circ\text{C}$. High temperatures may increase MDMA and METH hyperthermia and may promote their toxic effects (Malberg and Seiden 1998; Haughey et al. 2000; Pubill et al. 2003). Moreover, such temperatures simulate the hot temperatures typical of the nightclubs in which MDMA and METH are consumed. Background masking noise was supplied by a radio playing at low volume. Rats were placed in the chamber 30 min prior to the first drug injection and were injected once every 2 h from then on for a total of four injections. They remained in the test environment for 1 h after the last injection (total time in chamber = 7 h 30 min).

Body temperature was measured as previously described (McGregor et al. 2003b) using a Braun Thermoscan Instant thermometer (IRT 1020) inserted into the ear of the rat. These measurements were taken immediately prior to each injection and 1 h after the last injection.

Head-weaving was assessed as number of head weaves (back and forth once) (Wallace et al. 1999). This was counted during a 1-min period at the end of testing (1 h after the final injection).

At the conclusion of testing, rats were individually housed overnight to minimise any risk of fighting or overheating, and were then returned to group housing the next morning.

Social interaction test

Four weeks after dosing, rats were tested in the social interaction test as described previously (McGregor et al. 2003a). Testing took place in a different room to that in which acute drug administration had occurred. The test arena consisted of a black square Perspex box ($52 \times 52 \times 40$ cm) dimly lit by a red 40 W light bulb. A miniature infrared video camera was mounted above the centre of the box and this sent video pictures to a monitor and VCR in a neighbouring room in which behaviour was recorded and scored by an experimenter blind to treatment. Background noise was provided by a radio at low volume and the arena wiped down with 50% ethanol between each test pair.

Testing was conducted on 3 consecutive days, with each rat tested once per day, in a trial lasting 10 min. For each of three trials, each rat was paired with a different partner of similar weight and same treatment group, but from a different home cage. The duration of interaction was recorded by an experimenter in an adjoining room using ODLog software (<http://www.macropodsoftware.com>). Behaviours recorded included sniffing, following, mutual grooming and crawling under/over.

Emergence test

One week after social interaction rats were tested in the emergence test as previously described (McGregor et al. 2003a). Testing took place in a different room to that in which acute drug administration had occurred. The test arena consisted of black Perspex walls surrounding a white Perspex floor ($96 \times 100 \times 40$ cm) with a black wooden hide box ($24 \times 40 \times 15$ cm, with hinged lid) placed in the top right corner of the arena. The arena was illuminated with a 60 W white light bulb mounted with a miniature video camera approximately 1 m above the centre of the arena. Background noise was provided by radio at low volume and the arena wiped down with 50% ethanol in between each trial.

Testing began with the rat being placed in the hide box. Behaviour was recorded for 5 min by an experimenter blind to group assignment viewing a monitor in an adjacent room. Behaviour measures recorded included (1) latency: the amount of time taken for the rat to completely emerge from the hide box for the first time, (2) risk assessment: the total time the rat spent with its head poking out of the hide box but the hind legs remaining inside the box, and

(3) open field time: the total time spent entirely out of the hide box. Data were recorded using ODLog data logging software from Macropod software (<http://www.macropodsoftware.com>).

Neurochemical analysis

Seven weeks following drug treatment rats were rapidly decapitated and their brains removed. As described previously (McGregor et al. 2003b), brains were dissected by hand into five regions of interest (hypothalamus, striatum, prefrontal cortex, hippocampus and amygdala) over dry ice and frozen at -80°C until analysis.

Tissue was weighed and homogenised in 500 μl of 0.2 M perchloric acid containing 0.1% cysteine and 200 nM of internal standard (5-hydroxy-N-methyl-tryptamine; 5-HMeT) using a glass-Teflon homogeniser and Brinkman polytron. Homogenate was then centrifuged for 10 min at 15,000 g, 4°C and pellet discarded. A 20 μl aliquot was then analysed for bioamine content using HPLC with electro-chemical detection.

Briefly, the HPLC system consisted of a Shimadzu ADVP module (Kyoto, Japan) equipped with SIL-10 autoinjector with sample cooler and LC-10 on-line 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), PIC B-8 octane sulphonic acid (Waters, Australia) 0.74 mmol/l, sodium EDTA (0.3 mmol/l) and methanol (10% v/v). The flow rate was maintained at 0.85 ml/min.

Chromatographic separation of dopamine, dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), 5-HT and 5-HMeT were accomplished on a Merck LiChrospher 100 RP-18 reversed phase column maintained at 27.5°C . Quantification was achieved via an INTRO electrochemical detector (Antec Leyden, Netherlands) equipped with Faraday-shielded oven compartment and a glassy carbon working electrode set at $+0.75$ V. The concentrations of unknown samples were obtained from the linear regression equation of calibration curves by plotting concentration versus area ratio of the external standard and internal standard.

Statistics

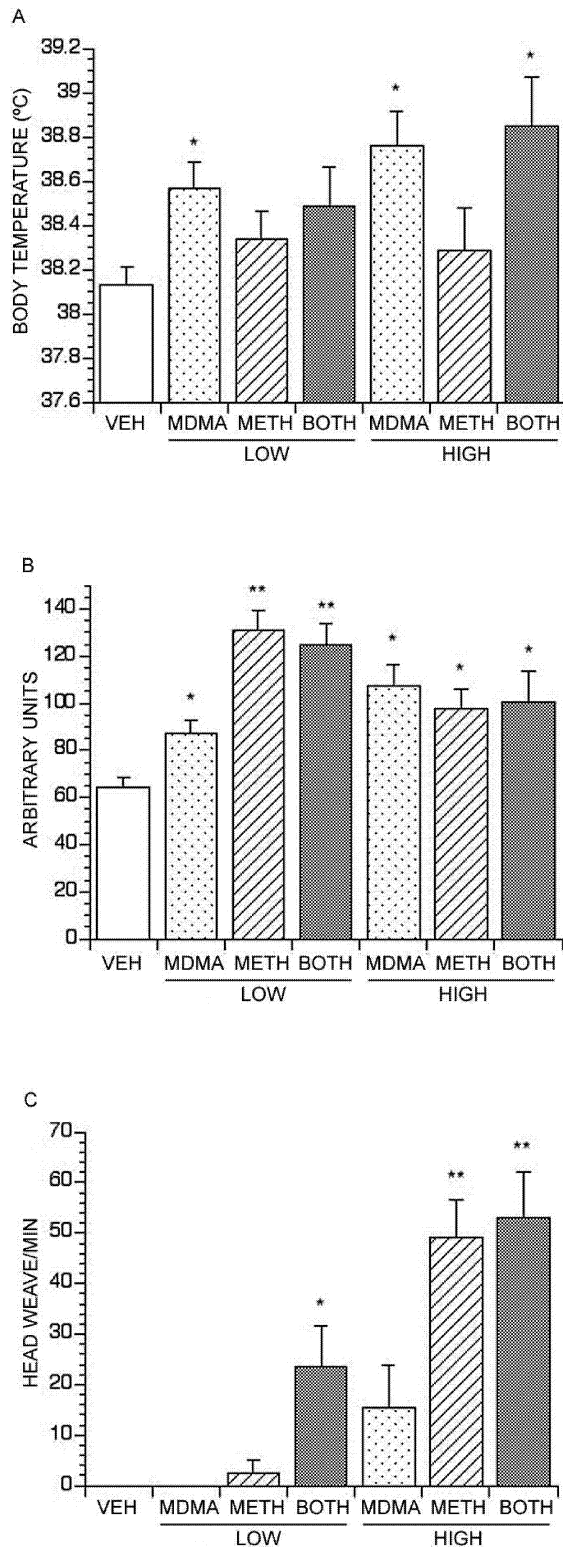
All data were analyzed using a priori contrasts (Minium et al. 1993) in the form of one-way analysis of variance (ANOVA) comparing each treatment group with the vehicle group. Levene's test was used to ensure homogeneity of variance. Neurochemical data were statistically analyzed as ng/g tissue but for ease of exposition are reported as percentage change from vehicle group mean. Differences were considered significant when $P < 0.05$.

Results

Acute drug effects

The mean maximum temperature for each group reached over the 7.5 h drug administration period is shown in Fig. 2A. Significant hyperthermic responses were observed in the low and high MDMA groups and the high MDMA/METH group [$F(6,54) = 4.28; 8.42; 10.92, P < 0.05$].

Total locomotor activity for each group over the 7.5 h drug administration phase is shown in Fig. 2B. All drug treated rats displayed significantly greater total locomotor activity than controls. The low METH and low MDMA/METH groups exhibited the greatest locomotor activity over the test period [$F(6,54) = 34.70; 28.59, P < 0.001$]. The



slightly lower locomotor activation in the high METH and high MDMA/METH groups was associated with increased head-weaving in these groups [$F(6,50)=31.23$; 36.35 , $P<0.001$, Fig. 2C]. Video monitoring of behaviour showed that forward locomotor activity decreased in these groups as the repetitive movements of the head and front paws

Fig. 2 A Mean highest temperature recorded during the 7.5 h drug administration phase at a high ambient temperature (28°C). Bars represent mean temperature+SEM after vehicle, low or high METH, MDMA or MDMA/METH combination (BOTH). *Indicates significantly different from vehicle $P<0.05$, with 8–10 rats per group. **B** Total activity recorded over the 7.5 h drug administration phase as detected by infrared cameras and LabView software. Animals received four injections every 2 h after a 0.5-h habituation period, and remained in the activity chamber for 1 h after the last injection. Bars represent mean+SEM of arbitrary units. **C** Number of head-weaving movements counted over a 1-min period at the conclusion of the drug administration phase, 1 h after the last injection. Bars represent mean+SEM. * Indicates significantly different from vehicle $P<0.05$; ** indicates significantly different from vehicle $P<0.001$, with 8–10 rats per group

Table 1 Results from social interaction test. Values are mean (SEM) time in seconds spent interacting, with 8–10 pairs of rats per treatment group

Treatment group	Interaction time (SEM)
Vehicle	102.39 (13.27)
Low MDMA	88.36 (9.13)
Low METH	66.38 (7.85)**
Low MDMA/METH	63.49 (9.18)**
High MDMA	51.16 (7.47)***
High METH	58.09 (9.23)**
High MDMA/METH	55.48 (8.21)**

Asterisks indicate significant difference from vehicle at the ** $P<0.01$ and *** $P<0.001$ level

developed. Low MDMA/METH group rats also displayed significant head-weaving [$F(6,50)=7.11$, $P<0.05$]. Low MDMA and vehicle group rats did not show any head-weaving. The high MDMA/METH group showed the most severe reaction to treatment, with profuse sweating and porphyrin around the eyes and nose.

Social interaction test

Results of the social interaction test conducted 4 weeks following drug treatment are shown in Table 1. The high MDMA [$F(6,86)=14.17$, $P<0.001$], low and high METH [$F(6,86)=7.61$; 10.60 , $P<0.01$] and low and high MDMA/METH [$F(6,86)=8.87$; 11.99 , $P<0.01$] groups all displayed a significant reduction in social interaction time compared to controls. The low MDMA group rats were not significantly different to controls ($P=0.29$).

Emergence test

The results for the emergence test are shown in Table 2. The low MDMA group and both the low and high MDMA/METH groups showed a reduction in open field time relative to controls [$F(6,54)=5.15$; 4.44 ; 4.83 , $P<0.05$] and increased time spent in risk assessment [$F(6,54)=7.46$; 5.36 ; 4.84 , $P<0.05$]. The high MDMA group also showed a trend towards increased risk assessment that

Table 2 Results from the emergence test. Results represent mean (SEM) in seconds, with 8–10 animals per treatment group. See Materials and methods for description of behavioural measures

Treatment	Latency	Risk assessment	Open field
Vehicle	33.79 (7.12)	46.77 (4.25)	113.74 (14.53)
Low MDMA	79.74 (29.27)	81.13 (11.96)*	52.54 (20.15)*
Low METH	59.71 (29.99)	53.30 (9.08)	131.13 (20.59)
Low MDMA/METH	98.33 (35.81)	76.80 (7.71)*	54.36 (17.73)*
High MDMA	78.48 (33.68)	69.83 (8.62)	95.91 (23.90)
High METH	36.15 (4.86)	52.09 (11.17)	136.91 (25.01)
High MDMA/METH	75.83 (35.51)	75.35 (11.36)*	52.69 (17.26)*

*Indicates significantly different from vehicle, $P < 0.05$

was non-significant ($P = 0.08$). There were no significant differences in emergence latency between groups, although a trend for increased emergence latency in both MDMA and both MDMA/METH groups was evident.

Neurochemical analysis

Results of the neurochemical analysis are shown in Table 3. The low MDMA group showed a significant decrease in 5-HT and 5-HIAA in the hippocampus [$F(6,50) = 5.22; 4.34, P < 0.05$], while the high MDMA group showed marked decreases of 5-HT and 5-HIAA in the prefrontal cortex [$F(6,50) = 7.46; 6.23, P < 0.001$], striatum [$F(6,52) = 12.84; 13.56, P < 0.001$] and hippocampus [$F(6,50) = 18.42; 17.36, P < 0.001$] and 5-HT in the amygdala [$F(6,52) = 7.24, P < 0.01$].

The low METH group showed reduced dopamine levels in the prefrontal cortex [$F(6,48) = 5.56, P < 0.05$] and the high METH group displayed decreased dopamine and DOPAC in this region [$F(6,48) = 6.94; 4.92, P < 0.05$].

No significant neurochemical changes were detected in the low MDMA/METH group. However, high MDMA/METH treatment led to decreased 5-HT in the prefrontal cortex [$F(6,50) = 4.63, P < 0.05$], decreased dopamine, DOPAC, 5-HT and 5-HIAA in the striatum [$F(6,52) = 8.42; 7.94; 8.30; 8.47, P < 0.01$], and decreased 5-HT and 5-HIAA in the hippocampus [$F(6,50) = 10.38; 5.90, P < 0.05$]. No changes in dopamine and DOPAC in the amygdala or hippocampus were detected.

No neurochemical changes were detected in the hypothalamus of any group (data not shown).

Discussion

The present results show that co-administration of MDMA and METH leads to adverse effects that may be greater than those observed with similar doses of MDMA or METH administered alone. These results also demonstrate specific adverse behavioural and neurochemical effects of MDMA and METH given alone at relatively low doses in

Table 3 Percent of vehicle group [mean (SEM)] for DA, DOPAC, 5-HT and 5-HIAA in various brain regions. $n = 8-10$ rats per group. Mean values for vehicle group in ng/g tissue of DA, DOPAC, 5-HT and 5-HIAA, respectively, are: prefrontal cortex: 317.1, 24.2, 269.2, 92.5; striatum: 11079.1, 787.1, 584.1, 400.4; hippocampus: 28.3, 4.8, 501.4, 295.6; amygdala: 315.2, 23.2, 795.1, 258.5. DA dopamine; DOPAC dihydroxyphenylacetic acid; 5-HT 5-hydroxytryptamine; 5-HIAA 5-hydroxyindolacetic acid

Region	Low MDMA	Low METH	Low MDMA METH	High MDMA	High METH	High MDMA METH
<i>Prefrontal cortex</i>						
DA	77.8 (22.2)	54.3 (12.0)*	64.0 (22.5)	78.5 (16.8)	47.1 (23.1)*	64.8 (24.1)
DOPAC	94.2 (17.5)	71.5 (7.5)	76.7 (10.4)	88.8 (11.1)	63.6 (14.3)*	82.1 (16.8)
5-HT	90.0 (3.9)	99.0 (6.17)	93.0 (4.0)	82.9 (7.3)*	91.9 (3.9)	86.0 (4.0)*
5-HIAA	95.6 (4.9)	99.4 (6.1)	98.7 (2.4)	84.2 (6.6)*	89.4 (5.6)	90.2 (4.0)
<i>Striatum</i>						
DA	88.6 (9.7)	84.2 (7.6)	89.4 (8.5)	84.0 (8.4)	92.9 (10.7)	69.6 (11.0)**
DOPAC	87.1 (8.8)	86.8 (6.7)	92.7 (7.1)	81.3 (8.1)	95.5 (8.9)	73.4 (9.3)**
5-HT	83.1 (8.6)	89.8 (9.5)	101.9 (7.0)	63.2 (8.2)***	91.1 (9.8)	70.4 (11.9)**
5-HIAA	82.8 (7.2)	85.2 (7.8)	97.3 (6.6)	66.0 (8.1)***	93.7 (9.2)	73.1 (11.0)**
<i>Hippocampus</i>						
DA	102.6 (13.6)	93.6 (15.6)	88.7 (13.7)	109.7 (17.8)	100.0 (9.6)	93.0 (14.3)
DOPAC	115.4 (9.4)	107.4 (11.9)	94.4 (6.6)	106.5 (14.0)	118.3 (5.7)	110.0 (6.5)
5-HT	87.2 (3.6)*	94.9 (4.0)	92.4 (4.2)	70.8 (8.0)***	99.2 (2.7)	78.9 (8.5)*
5-HIAA	82.3 (6.4)*	87.4 (5.9)	93.3 (6.3)	65.6 (8.5)***	90.4 (4.7)	80.6 (9.0)*
<i>Amygdala</i>						
DA	103.3 (13.2)	113.6 (11.7)	88.9 (10.5)	89.2 (7.3)	88.8 (17.1)	82.5 (10.3)
DOPAC	111.0 (10.8)	128.9 (11.8)	107.2 (10.7)	104.3 (13.8)	107.2 (14.3)	94.8 (8.5)
5-HT	94.9 (3.9)	103.6 (2.3)	101.8 (3.7)	84.2 (7.1)**	104.0 (2.7)	92.0 (3.5)
5-HIAA	106.3 (9.3)	107.6 (4.2)	106.1 (4.0)	83.9 (7.0)	103.2 (5.0)	91.3 (4.8)

Asterisks indicate significantly different from vehicle group mean (100%) at the * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ levels of significance

a novel 1-day, multiple injection procedure. Of particular note is the marked decrease in social interaction 4 weeks after brief multiple low dose METH administration.

The acute hyperthermic response seen here in both MDMA groups has been widely reported by our group and many others (Gurtman et al. 2002; Green et al. 2003; McGregor et al. 2003b) and has been linked to neurotoxicity (Malberg and Seiden 1998). Conversely, neither of the groups given only METH showed hyperthermia. Previous studies showing hyperthermia with METH in rats have used notably higher doses than those used here (Haughey et al. 2000; Brown et al. 2003).

Interestingly MDMA/METH co-administration caused a robust hyperthermic effect, with the high MDMA/METH group rats exhibiting the highest temperatures of any of the groups studied, despite receiving 60% less MDMA than the high dose MDMA group. This indicates that METH can exacerbate the acute hyperthermia seen with MDMA, a hypothesis that is further supported by the severe hyperthermic response seen in our initial pilot studies using 2.5 (MDMA) +2.5 (METH) mg/kg dose regime. This suggests humans using MDMA/METH combinations might be particularly susceptible to adverse hyperthermic reactions at high ambient temperatures.

The acute locomotor hyperactivity seen in all drug treatment groups is consistent with previous reports of MDMA and METH stimulant effects (Wallace et al. 1999; Gurtman et al. 2002; McGregor et al. 2003b). The lower counts evident in the high METH and high MDMA/METH groups reflect high levels of head-weaving behaviour in these rats, typical of moderate to high doses of METH (Wallace et al. 1999). Interestingly, when METH was given with MDMA, less than half of the dose of METH produced the same amount of head-weaving. Again, an interactive effect of MDMA and METH is suggested.

The decreased social interaction seen 4 weeks after MDMA treatment confirms several previous reports of this phenomenon (Morley et al. 2001; Fone et al. 2002; Gurtman et al. 2002; Bull et al. 2003; McGregor et al. 2003a, 2003b). Interestingly, in the present study the same phenomenon was also seen in rats given either METH or a MDMA/METH combination. While decreased social interaction during acute METH administration has been reported in rodents (Shinba et al. 1996), to our knowledge this is the first report of long term decreases in social interaction following METH administration. Paranoia, anxiety and social withdrawal are well documented phenomena in human METH users (Davidson et al. 2001; Rawson et al. 2002) and the effects seen here in rats may reflect this phenomenon. It is particularly interesting that a relatively low dose regime of METH (4×2.5 mg/kg) can have such a lasting effect on social behaviour.

There was some disparity between results from the social interaction and emergence tests of anxiety. While every group except the low MDMA group showed decreased social interaction, changes in emergence behaviour were only seen in the two MDMA/METH groups and the low MDMA group, although the high MDMA group

effect approached significance. This disparity suggests that distinct mechanisms may be involved in these two tests of anxiety, with METH treated groups exhibiting increased social anxiety but no increases in the generalised anxiety that the emergence test is thought to measure. A similar disparity has previously been observed in our laboratory using these two tests. For example, acute MDMA treatment appears to decrease social anxiety in rats but is strongly anxiogenic in the emergence test (Morley and McGregor 2000). It is likely then that these two tests assess separate facets of anxiety that may be differentially susceptible to different drug treatments.

An important finding was that both of the MDMA/METH groups were affected on both the social interaction and the emergence tests. Of particular note is the decreased social interaction and increased emergence-related anxiety measures in the low MDMA/METH group. At doses directly comparable to human use (1.25+1.25 mg/kg, four doses, 2 h inter-dose interval) this is particularly relevant, and may model the long-term behavioural changes reported in human MDMA/METH users (Brecht and von Mayrhauser 2002).

Neurochemical analysis revealed that MDMA treatment decreased 5-HT and 5-HIAA in four brain regions as reported previously by our group and many others (Gurtman et al. 2002; Green et al. 2003; McGregor et al. 2003a, 2003b). The fact that decreased 5-HT and 5-HIAA was seen in the hippocampus of the low MDMA group suggests a particular vulnerability of this brain region to MDMA neurotoxicity.

Given alone, METH decreased dopamine in the prefrontal cortex at both dose levels. Evidence of prefrontal depletion of dopamine at the relatively low dose of 4×2.5 mg/kg IP is a novel finding and indicates lasting adverse neurochemical effects of relatively small amounts of METH. In combination with the social changes evident in this group, this may have implications for future human studies.

The lack of striatal dopamine depletion in the presence of prefrontal depletion in the METH groups is notable. Striatal dopamine depletion has been reported at various intervals after high dose METH treatment (Fleckenstein et al. 2000; Brown et al. 2003) although not with lower dose regimes such as those used here (e.g. 4×5 mg/kg) (Chapman et al. 2001). Therefore results here may indicate a pronounced susceptibility of prefrontal neurons to METH toxicity. Alternatively, these results might reflect greater recovery of striatal than prefrontal dopamine neurons following low dose METH administration (Cass and Manning 1999; Pubill et al. 2003).

The significant degree of monoamine depletion evident in the high MDMA/METH group indicates a greater likely neurotoxic effect of MDMA and METH combinations than either drug alone. Decreases in 5-HT and 5-HIAA in the prefrontal cortex, striatum and hippocampus suggests 5-HT neurotoxicity, despite the dose of MDMA administered (4×2 mg/kg) being far less than that of the low MDMA group which showed decreased 5-HT and 5-HIAA in the hippocampus alone. Both MDMA and

METH administration can result in long-term 5-HT depletion (Chapman et al. 2001; McGregor et al. 2003a), but at much higher doses than those used in combination here. Similarly, although the high MDMA/METH group did not show dopamine depletion in the prefrontal cortex like the METH groups, there was a clear and unique dopamine depleting effect in the striatum. This is despite the total METH dose given in this group (8 mg/kg) being far less than in the high METH group (20 mg/kg). This result is intriguingly similar to the finding of Reneman and colleagues (2002) that combined MDMA/METH users have decreased striatal dopamine transporter density compared to users of MDMA only.

The potentiating effect of MDMA on METH-induced dopamine depletion in the striatum may be linked to MDMA-induced dopamine release (Green et al. 2003). METH-induced dopamine depletion may be exaggerated by additional MDMA induced dopamine release that is not sufficient to induce long-term depletion alone. Clearly, in vivo microdialysis studies would be of value in further examining this hypothesis. On the other hand, serotonin release induced by METH may summate with that produced by MDMA to exacerbate MDMA serotonergic depletion. METH induced dopamine release may also have a role in this, given previous evidence that dopamine may play a role in promoting MDMA-induced serotonergic neurotoxicity (Stone et al. 1988; Schmidt et al. 1990; Shankaran et al. 1999). Further, interactions between MDMA and METH at a pharmacokinetic level are likely (Hansen et al. 2002) and may contribute to some of the effects reported here.

In summary, rats given the MDMA/METH combinations showed several significant adverse effects including high body temperature and head-weaving during acute drug administration and decreased social interaction, increased indications of anxiety on the emergence test and serotonergic and dopaminergic depletion several weeks later. These effects partly reflect a combination of the adverse effects seen with each drug individually: the head-weaving and dopamine depletion seen with METH and the hyperthermia, anxiety and serotonergic neurotoxicity seen with MDMA. However, the long-term adverse behavioural effects appeared more pronounced and consistent in rats given the MDMA/METH combination and the high MDMA/METH group displayed significant depletion of striatal dopamine that was not seen with either drug given alone. These results have potentially important implications for human harm minimisation and public awareness. As the nature of human drug use tends to be increasingly polymorphic, further studies examining the combined effects of common drugs of abuse appear especially relevant.

Acknowledgement This work was supported by an NH&MRC grant to Iain S. McGregor and Glenn E. Hunt.

References

- Australian Bureau of Criminal Intelligence, (2002) Australian Illicit Drug Report 2001–2002. Canberra, Commonwealth of Australia
- Bisagno V, Ferguson D, Luine VN (2002) Short toxic methamphetamine schedule impairs object recognition task in male rats. *Brain Res* 940:95–101
- Boys A, Lenton S, Norcross K (1997) Polydrug use at raves by a Western Australian sample. *Drug Alcohol Rev* 16:227–234
- Brecht ML, von Mayrhauser C (2002) Differences between ecstasy-using and nonusing methamphetamine users. *J Psychoact Drugs* 34:215–223
- Brown PL, Wise RA, Kiyatkin EA (2003) Brain hyperthermia is induced by methamphetamine and exacerbated by social interaction. *J Neurosci* 23:3924–3929
- Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, Wilke F, Wartberg L, Zapletalova P, Clausen M (2003) Long-term effects of “ecstasy” use on serotonin transporters of the brain investigated by PET. *J Nucl Med* 44:375–384
- Bull EJ, Hutson PH, Fone KCF (2003) Reduced social interaction following 3,4-methylenedioxymethamphetamine is not associated with enhanced 5-HT(2C) receptor responsiveness. *Neuropharmacology* 44:439–448
- Cass WA, Manning MW (1999) Recovery of presynaptic dopaminergic functioning in rats treated with neurotoxic doses of methamphetamine. *J Neurosci* 19:7653–7660
- Chapman DE, Hanson GR, Kesner RP, Keefe KA (2001) Long-term changes in basal ganglia function after a neurotoxic regimen of methamphetamine. *J Pharmacol Exp Ther* 296:520–527
- Davidson C, Gow AJ, Lee TH, Ellinwood EH (2001) Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Res Rev* 36:1–22
- Ernst T, Chang L, Leonido-Yee M, Speck O (2000) Evidence for long-term neurotoxicity associated with methamphetamine abuse: a 1H MRS study. *Neurology* 54:1344–1349
- Fleckenstein AE, Gibb JW, Hanson GR (2000) Differential effects of stimulants on monoaminergic transporters: pharmacological consequences and implications for neurotoxicity. *Eur J Pharmacol* 406:1–13
- Fone KCF, Beckett SRG, Topham IA, Swettenham J, Ball M, Maddocks L (2002) Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity. *Psychopharmacology* 159:437–444
- Fox HC, McLean A, Turner JJ, Parrott AC, Rogers R, Sahakian BJ (2002) Neuropsychological evidence of a relatively selective profile of temporal dysfunction in drug-free MDMA (“ecstasy”) polydrug users. *Psychopharmacology* 162:203–214
- Friedman SD, Castaneda E, Hodge GK (1998) Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav* 61:35–44
- Green AR, Mehan 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
- Gurtman CG, Morley KC, Li KM, Hunt GE, McGregor IS (2002) Increased anxiety in rats after 3,4-methylenedioxymethamphetamine: association with serotonin depletion. *Eur J Pharmacol* 446:89–96
- Hansen JP, Riddle EL, Sandoval V, Brown JM, Gibb JW, Hanson GR, Fleckenstein AE (2002) Methylenedioxymethamphetamine decreases plasmalemmal and vesicular dopamine transport: mechanisms and implications for neurotoxicity. *J Pharmacol Exp Ther* 300:1093–1100

- Hatzidimitriou G, McCann UD, Ricaurte GA (1999) Altered serotonin innervation patterns in the forebrain of monkeys treated with (\pm)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. *J Neurosci* 19:5096–5107
- Haughey HM, Fleckenstein AE, Metzger RR, Hanson GR (2000) The effects of methamphetamine on serotonin transporter activity: role of dopamine and hyperthermia. *J Neurochem* 75:1608–1617
- Kita T, Wagner GC, Nakashima T (2003) Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. *J Pharmacol Sci* 92:178–195
- Malberg JE, Seiden LS (1998) Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J Neurosci* 18:5086–5094
- McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA (1998) Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11 C]WIN-35,428. *J Neurosci* 18:8417–8422
- McCann UD, Mertl M, Eligulashvili V, Ricaurte GA (1999) Cognitive performance in (\pm) 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) users: a controlled study. *Psychopharmacology* 143:417–425
- McGregor IS, Clemens KJ, Van Der Plasse G, Li KM, Hunt GE, Chen F, Lawrence AJ (2003a) Increased anxiety 3 months after brief exposure to MDMA (“ecstasy”) in rats: association with altered 5-HT transporter and receptor density. *Neuropsychopharmacology* 28:1472–1484
- McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM, Cornish JL, Hunt GE (2003b) Increased anxiety and “depressive” symptoms months after MDMA (“ecstasy”) in rats: drug-induced hyperthermia does not predict long-term outcomes. *Psychopharmacology* 168: 465–474
- Minium EW, King BM, Bear G (1993) *Statistical reasoning in psychology and education*, 3rd edn. Wiley, New York
- Morley KC, McGregor IS (2000) (\pm)-3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) increases social interaction in rats. *Eur J Pharmacol* 408:41–49
- Morley KC, Gallate JE, Hunt GE, Mallet PE, McGregor IS (2001) Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine (“ecstasy”). *Eur J Pharmacol* 433:91–99
- Pubill D, Canudas AM, Pallas M, Camins A, Camarasa J, Escubedo E (2003) Different glial response to methamphetamine- and methylenedioxymethamphetamine-induced neurotoxicity. *Nauyn-Schmiedeberg's Arch Pharmacol* 367:490–499
- Rawson RA, Gonzales R, Brethen P (2002) Treatment of methamphetamine use disorders: an update. *J Subst Abuse Treat* 23:145–150
- Reneman L, Enderet E, de Bruin K, Lavalaye J, Feenstra MG, de Wolff FA, Booij J (2002) The acute and chronic effects of MDMA (“ecstasy”) on cortical 5-HT $_{2A}$ receptors in rat and human brain. *Neuropsychopharmacology* 26:387–396
- Sabol KE, Lew R, Richards JB, Vosmer GL, Seiden LS (1996) Methylenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part I: synaptosomal uptake and tissue concentrations. *J Pharmacol Exp Ther* 276:846–854
- Schmidt CJ, Black CK, Taylor VL (1990) Antagonism of the neurotoxicity due to a single administration of methylenedioxymethamphetamine. *Eur J Pharmacol* 181:59–70
- Shankaran M, Yamamoto BK, Gudelsky GA (1999) Mazindol attenuates the 3,4-methylenedioxymethamphetamine-induced formation of hydroxyl radicals and long-term depletion of serotonin in the striatum. *J Neurochem* 72:2516–2522
- Shinba T, Yamamoto K, Cao GM, Mugishima G, Andow Y, Hoshino T (1996) Effects of acute methamphetamine administration on spacing in paired rats: investigation with an automated video-analysis method. *Prog Neuropsychopharmacol Biol Psychiatry* 20:1037–1049
- Stone DM, Johnson M, Hanson GR, Gibb JW (1988) Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *J Pharmacol Exp Ther* 247:79–87
- Stuerenburg HJ, Petersen K, Baumer T, Rosenkranz M, Buhmann C, Thomasius R (2002) Plasma concentrations of 5-HT, 5-HIAA, norepinephrine, epinephrine and dopamine in ecstasy users. *Neuroendocrinol Lett* 23:259–261
- Thomasius R, Petersen K, Buchert R, Andresen B, Zapletalova P, Wartberg L, Nebeling B, Schmoldt A (2003) Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users. *Psychopharmacology* 167:85–96
- Topp L, Hando J, Dillon P, Roche A, Solowij N (1999) ecstasy use in Australia: patterns of use and associated harm. *Drug Alcohol Depend* 55:105–115
- Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J (2001) Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J Neurosci* 21:9414–9418
- Wallace TL, Gudelsky GA, Vorhees CV (1999) Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. *J Neurosci* 19:9141–9148

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.