

ORIGINAL INVESTIGATION

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Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat

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Abstract *Rationale:* A variety of animal models have shown MDMA (3,4-methylenedioxyamphetamine) to be a selective 5-HT neurotoxin, though little is known of the long-term behavioural effects of the pathophysiology. The widespread recreational use of MDMA thus raises concerns over the long-term functional sequelae in humans. *Objective:* This study was designed to explore both the acute- and post-treatment consequences of a 3-day neurotoxic exposure to MDMA in the rat, using a variety of behavioural paradigms. *Methods:* Following training to pretreatment performance criteria, animals were treated twice daily with ascending doses of MDMA (10, 15, 20 mg/kg) over 3 days. Body temperature, locomotor activity, skilled paw-reaching ability and performance of the delayed non-match to place (DNMTP) procedure was assessed daily during this period and on an intermittent schedule over the following 16 days. Finally, post mortem biochemical analyses of [³H] citalopram binding and monoamine levels were performed. *Results:* During the MDMA treatment period, an acute 5-HT-like syndrome was observed which showed evidence of tolerance. Once drug treatment ceased the syndrome abated completely. During the post-treatment phase, a selective, delay-dependent, deficit in DNMTP performance developed. Post-mortem analysis confirmed reductions in markers of 5-HT function, in cortex, hippocampus and striatum. *Conclusions:* These results confirm that acutely MDMA exposure elicits a classical 5-HT syndrome. In the long-term, exposure results in 5-HT neurotoxicity and a lasting cognitive impairment. These results have significant implications for the prediction that use of MDMA in humans could have deleterious long-term neuropsychological/psychiatric consequences.

Key words MDMA · Cognition · DNMTP · Paw-reaching · Locomotor activity · Temperature · 5-HT · Citalopram binding · Neurotoxicity

Introduction

MDMA (3,4-methylenedioxyamphetamine), or “Ecstasy”, is a widely used recreational drug of abuse that is often perceived as “safe”, by its users (Peroutka 1987; Randall 1992; McDowell and Kleber 1994; Parrott 1998). However, recent evidence has suggested that users suffer both long-term cognitive impairments (Parrott and Lasky 1998) and reductions in the number of brain 5-HT transporter sites (McCann et al. 1998). In animal studies MDMA has been shown to be selectively neurotoxic to 5-HT neurones (Battaglia et al. 1987a), while sparing dopamine neurones (Nash and Brodtkin 1991). The regional activity of tryptophan hydroxylase and corresponding concentrations of 5-HT and 5-HIAA are dramatically reduced 3 h after a single injection of MDMA (Stone et al. 1986). Similarly, 5-HT high-affinity uptake sites are concomitantly decreased in number (Battaglia et al. 1987b). Multiple injections of MDMA result in a persistent depletion of these serotonergic parameters for at least 3 weeks after treatment (Stone et al. 1987). This possible prolonged depletion of 5-HT systems by MDMA is of particular concern in view of the widespread abuse of this compound. Re-innervation of 5-HT terminal regions has been reported both in rats (Battaglia et al. 1987a; Molliver et al. 1990; Ricaurte et al. 1992; Scanzello et al. 1993; McNamara et al. 1995) and primates (Fischer et al. 1995) following MDMA exposure. Both clinical depression and age-related cognitive impairments (Fischer et al. 1995) are associated with reductions in 5-HT markers and may be amenable to treatment with drugs acting through the 5-HT system. Therefore, an improved understanding of the long-term consequences of MDMA exposure is of significant interest.

To date, most animal studies on MDMA have focused primarily on the physiological and behavioural changes

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associated with the acute phase of MDMA action. Acute administration typically produces increases in locomotion (characteristic of dopaminergic stimulation) and a behavioural syndrome characteristic of increased 5-HT receptor activity. This syndrome includes adoption of a low body posture, splayed hind limbs, forepaw treading, salivation and piloerection (Hiramatsu et al. 1988). Few studies have attempted to relate the chronic consequences of MDMA exposure to cognitive impairment. Pigeons exhibit a decrease in accuracy and response rates in a delayed non-matching to sample task immediately following MDMA treatment, but show no deficit in performance following chronic exposure (LeSage et al. 1993). The choice accuracy of rats in a T-maze following chronic exposure of MDMA was unaffected (Ricaurte et al. 1993).

In this study, the behavioural effects of MDMA across both the acute and chronic periods was addressed in the rat using three behavioural paradigms: i) measurement of spontaneous locomotor activity using a photocell cage system; ii) skilled motor function assessed by a skilled paw reach task and iii) cognition assessed by performance of an operant delayed non-match to place procedure. Monoamine levels and 5-HT uptake site density were determined 20 days after initial MDMA exposure, by HPLC analysis and [³H] citalopram binding, respectively.

Materials and methods

Animals and experimental design

Eighteen Male Lister Hooded rats (Charles River, Kent, UK) weighing 150–180 g at the beginning of the experiment were group housed in clear polycarbonate cages (40×25×20 cm). The animals were maintained at an ambient temperature of 20±1°C, with a 12-h light (7 a.m. to 7 p.m.)/dark cycle. Water was available ad libitum but food was restricted initially to 85% of their free feeding weight and then titrated to allow a weight gain of approximately 3–4 g per week. All procedures were performed in accordance with “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) and as prescribed by Home Office project licence 60/01552 according to the UK Animals (Scientific Procedures) Act 1986.

Animals were trained to perform both the “staircase” and the delayed non-matching to place (DNMTP) tasks to pre-determined criteria (see below), and then randomly allocated to saline ($n=9$) or MDMA ($n=9$) treatment groups. Over 3 days preceding drug treatment the rats were habituated to IP injections and the locomotor apparatus (IP saline, 30-min locomotor assessment). Racemic MDMA HCl (Sigma) in saline, or vehicle alone, was then administered IP twice daily (0800 and 1800 hours) over 3 consecutive days. The dose increased daily; 10, 15 and 20 mg/kg according to a protocol previously found to produce neurotoxic damage (Sharky et al. 1991). Immediately after the morning administration, locomotor activity was assessed for 30 min. Rectal temperature was then recorded prior to the staircase test (5 min), which was followed by the 40-min DNMTP test starting between 45 and 130 min after drug administration. A second staircase test was made between 1600 and 1700 hours prior to the second MDMA administration. After the drug treatment phase, testing was performed as laid out in Table 1. The animals were not tested every day to reduce the impact of over-training. Following completion of behavioural testing, 20 days after the first drug administration, the ani-

Table 1 Experimental design: illustration of the order (rows) and nature of the drug treatments of MDMA and the behavioural test performed (*shaded box*) on each day of the study. The animals were killed 1 day after testing was complete. *DNMTP* Delayed non-match to place

Reference	Pre	1	2	3	6	7	9	10	13	14	15	16	19
Week Day	Tu	W	Th	F	M	Tu	Th	F	M	Tu	Th	F	M
MDMA a.m. (mg)		10	15	20									
Locomotor		■	■	■	■	■	■	■	■	■	■	■	■
Temperature		■	■	■	■	■	■	■	■	■	■	■	■
Paw-reach a.m.		■	■	■	■	■	■	■	■	■	■	■	■
DNMTP		■	■	■	■	■	■	■	■	■	■	■	■
Paw-reach p.m.		■	■	■	■	■	■	■	■	■	■	■	■
MDMA p.m. (mg)		10	15	20									

imals were killed and brain tissue was collected for biochemical analysis and assay of transporter sites.

Behavioural procedures

Spontaneous behaviour and physiology

From the last pre-drug day to 5 days after the completion of the drug phase, regular observations of the general status of the animals were made. Locomotor activity was determined using eight arenas, each consisting of a clear polycarbonate cage of the same type to those used in the home cages but fitted with wire grid floors (RS Biotech, Alva, Clackmananshire, UK). Each was monitored by two infrared photo-beams positioned 4 cm from each end and 2.2 cm above the floor. One locomotor count was recorded if a rat interrupted both beams consecutively. Locomotor counts were recorded by a computer (Acorn A5000) programmed using the Arachnid control language. Rectal temperature was determined by inserting a thermocouple probe (BDH, Electronic thermometer, model 0390) approximately 5.0 cm past the anal sphincter.

Skilled paw-reach – “staircase task”

Six “staircase” test boxes constructed (Department of Pharmacology, University of Edinburgh, Edinburgh, UK) as previously described (Marston et al. 1995) were used to determine the level of skilled paw use. At the start of each test, an animal was introduced via the opening in the base of the box. The rat was then required to recover two reinforcement pellets (45 mg formula P precision reward pellets; Noyes Inc., New Hampshire, USA) from each of the six steps on the two stairs arranged either side of the central plinth. The box was constructed so that the rat was able to recover pellets from the left stair only with its left paw and visa versa for the right paw. The test was halted when either all the pellets had been recovered or 5 min had elapsed, whichever was sooner. Performance was scored by counting both the number of pellets successfully recovered and the number of pellets displaced, but not recovered. The pre-operative criterion was set at a minimum of nine pellets recovered from each stair in 5 min.

Delayed non-matching to position (DNMTP)

Six operant chambers (model E10809, Coulbourn Instruments, Pennsylvania, USA) were used, each interfaced to a RISC com-

puter programmed using the Arachnid on-line control language (Paul Fray Ltd, Cambridge, UK). Each box was equipped with a house light, two retractable levers and a central food magazine, access to which was monitored by an infrared beam. To start a trial one of the two levers, chosen at random, was presented as the "sample". Following a lever press the sample lever was retracted and the delay, chosen at random from 0.3, 1, 3, 5.6, 10, 17 or 30 s, initiated. During the delay, nose poke responses in the central food magazine were encouraged, the first such response following the completion of the delay triggered the presentation of both levers for the "choice". The animal was rewarded with a pellet in the magazine for responding on the lever opposite to that presented as the sample (correct trial). If a "same" lever press was made, the house light was extinguished and no reward given. Parameters of performance recorded included; number of trials completed, correct trials, beam breaks (magazine responses during delay) and response latency (final beam break to choice response) across each delay interval. Beam break rate was calculated as:

$$\frac{(\text{Beam breaks per delay}) - 1}{\text{Delay}}$$

The last break was subtracted as this was obligate and by definition performed after the completion of the programmed delay. Consequently, animals were only capable of performing one response at the shortest delay (0.3 s) and rarely more than two on 1-s trials. Therefore, analysis of beam break rate was restricted to data collected from the five longest delays. The pre-operative criterion was set at a minimum 85% correct (arcsine 67.2), over all delays, maintained for three sessions.

Biochemical analysis

Twelve rats (six control, six MDMA) were killed by decapitation, 20 days after the initiation of drug treatment, the brain being rapidly removed and placed on an ice-cold metal plate. The neocortex, hippocampus, and striatum were removed bilaterally by free-hand dissection and weighed. One of the paired neocortex, hippocampus and striata samples from each rat was placed in an Eppendorff tube and stored at -70°C until used in quantification of monoamine and monoamine metabolite concentrations. The other half of each pair was prepared for ligand binding studies.

[^3H] citalopram binding

Membranes were prepared by placing each dissected brain in 50 vol ice-cold 50 mM TRIS HCl (pH 7.4) 120 mM NaCl, 5 mM KCl and homogenised for 30 s. The homogenate was spun at 19 500 rpm for 10 min at 4°C . The pellet was resuspended in a further 50 vol of the same buffer and centrifuged. The final pellet was resuspended in 25 vol of the same buffer and stored frozen at -20°C .

[^3H] citalopram was used to label 5-HT uptake sites (specific activity 85 Ci/mmol; New England Nuclear) according to the method of Lawrence et al. (1993). Immediately before use, all membrane suspensions were thawed at room temperature and diluted to 300 vol with 50 mM TRIS HCl (pH 7.4) at 25°C . Striata from similarly treated animals were paired to provide sufficient protein for the assay. Duplicate samples of membrane were incubated with 0.5 nM [^3H] citalopram in 50 mM TRIS HCl (as above) in the absence or presence of 0.03–100 nM unlabeled citalopram. Incubations were carried out for 60 min at 25°C . Incubations were terminated and binding measured exactly as described (Lawrence et al. 1993). Non-specific binding was defined by addition of 10 μM citalopram.

Mono-amine analysis

Tissue levels of 5-HT, 5-HIAA, DA, DOPAC, HVA and NA were measured by high performance liquid chromatography (HPLC) with electrochemical detection (BAS-4B detector). The remaining

frozen tissues were homogenised in 2 ml 0.6 M perchloric acid in Eppendorff tubes, spun at 1000 rpm for 2 min and 20 μl samples of the supernatant fractions were injected directly onto the column. The column (250 \times 4.6 mm C-18 ultrasphere) was eluted isocratically (1 ml/min) with a mobile phase consisting of 100 mM citrate/acetate buffer (pH 5.12; adjusted using NaOH) containing 100 $\mu\text{g}/\text{ml}$ octanesulphonic acid, 5% methanol and 2% tetrahydrofuran. Monoamines and their metabolites were quantified by measurement of peak heights and compared with those of standards of known concentration.

Statistical analysis

Statistics were carried out using G.B. Stat. (Microdynamics Inc., Silver Spring, Maryland, USA) and using SigmaStat (Jandel Scientific, Erkrath, Germany). Post hoc analyses of "all pairwise comparisons" were carried out using Scheffe's test. Dunn's method was used for comparison with the control group in analysis of the biochemical data. In all cases, conformity to normality and equali-

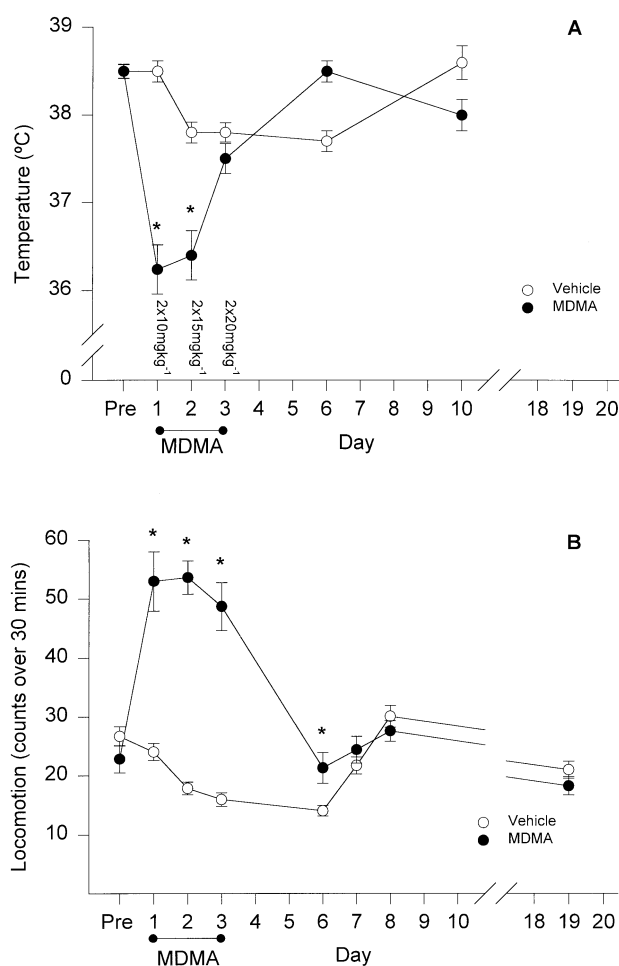


Fig. 1 **A** The effects of MDMA treatment across days 1–3, at the doses indicated, on rectal temperature over the period of the study (see Table 1). The data plotted represent mean and SEM for MDMA ($n=9$, ●) and vehicle ($n=9$, ○) treated groups. The bi-daily dosing regimen is annotated on the figure and represented by a bar, which is reproduced on the following figures. **B** Locomotor activity during the pretreatment, treatment and post-treatment phases of the experiment. MDMA exposure indicated by bar. The data plotted represent mean and SEM for MDMA ($n=9$) and vehicle ($n=9$) treated groups. * $P < 0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment

ty of variance was checked prior to parametric analysis. The percent correct data were subjected to an angular, or arcsine, transformation prior to analysis. Index Y was calculated according to the following equation (Sahgal 1987).

$$I_y = \frac{(\text{Left Correct} - \text{Right Correct})}{(\text{Left Correct} + \text{Right Correct})}$$

Results

Spontaneous behaviour and physiology

During the first 3–4 h following acute drug treatment, all of the MDMA treated rats exhibited one or more of the following behaviours: low body posture, splayed hind limbs, hyper-reactivity, piloerection and salivation. Analysis of the effect on core body temperature revealed an interaction between Treatment and Session [$F_{(5,96)}=12.14$, $P<0.005$] (Fig. 1A). Post hoc analysis confirmed that MDMA produced a significant hypothermic response over the first 2 days, but not day 3, in the acute drug treatment phase ($P<0.05$), after which body temperature was comparable with that of the saline controls. Analysis of the effect of MDMA on spontaneous locomotor output revealed an interaction between Treatment and Session [$F_{(2,671)}=26.31$, $P<0.001$] (Fig. 1B). Post hoc analysis confirmed that the level of locomotor activity of the MDMA treated rats was significantly greater than the saline controls during the acute treatment period, sessions 1–3 ($P<0.01$), and on post-drug test day 1 ($P<0.05$). Thereafter, activity scores were indistinguishable from control values.

Skilled paw-reaching

The performance of the rats was analysed according to four variables; test day or Session, Time of day (a.m. or

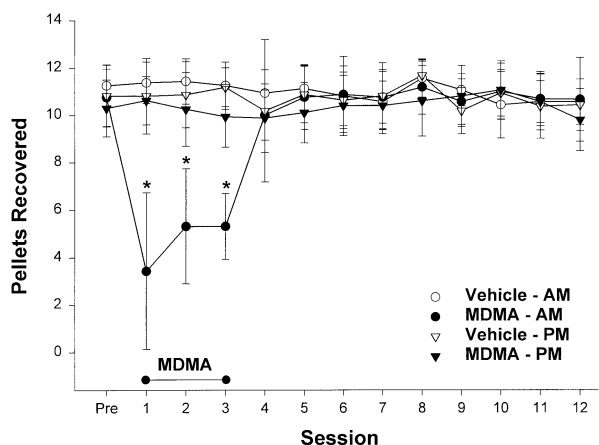


Fig. 2 The effects of MDMA exposure (indicated by bar) on the ability to perform the pre-learned skilled paw-reach task as indicated by the number of pellets recovered in the 5-min test sessions. Tests were performed twice a day (see Table 1). The data plotted represent mean and SEM for MDMA ($n=9$) and vehicle ($n=9$) treated groups. * $P<0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment. ○ Vehicle a.m., ● MDMA a.m., ▽ vehicle p.m., ▼ MDMA p.m.

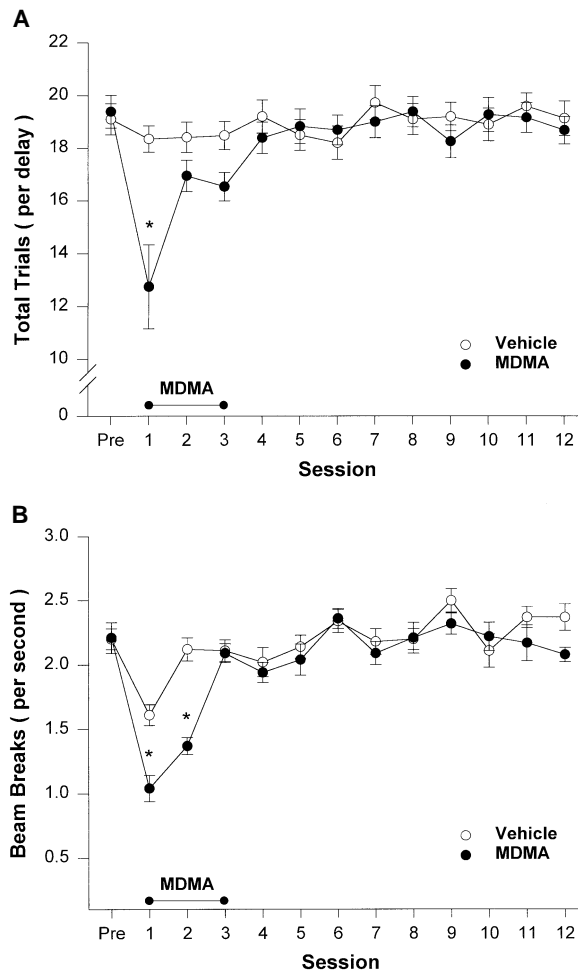


Fig. 3A, B Delayed non-match to place general performance. **A** Number of trials completed in each session across the three phases of the experiment. The data plotted represent mean and SEM for MDMA ($n=9$, ●) and vehicle ($n=9$, ○) treated groups. * $P<0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment. **B** Effect upon beam break rate (number of entries into food magazine during delay between cue and choice phases, divided duration of delay), before during and after MDMA exposure, indicated by bar. The data plotted represent mean and SEM for MDMA ($n=9$, ●) and vehicle ($n=9$, ○) treated groups. * $P<0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment

p.m.), Treatment and Side (left or right forepaw). An initial analysis of the side factor revealed no significant difference between the performance of the left and right forelimbs ($P>0.05$). The data for side was therefore pooled in all remaining analyses. ANOVA revealed main effects of Treatment, Time of day and Session with a significant three-way interaction between all the factors [$F_{(12,784)}=15.42$, $P<0.001$] (Fig. 2). Post hoc analysis confirmed that performance of the control group was consistent across both Session and Time of day. In the treatment period, group performance in the afternoon (p.m.) test never differed from control. By contrast, performance in the morning assessment, approximately 45 min after MDMA treatment, was significantly disrupted

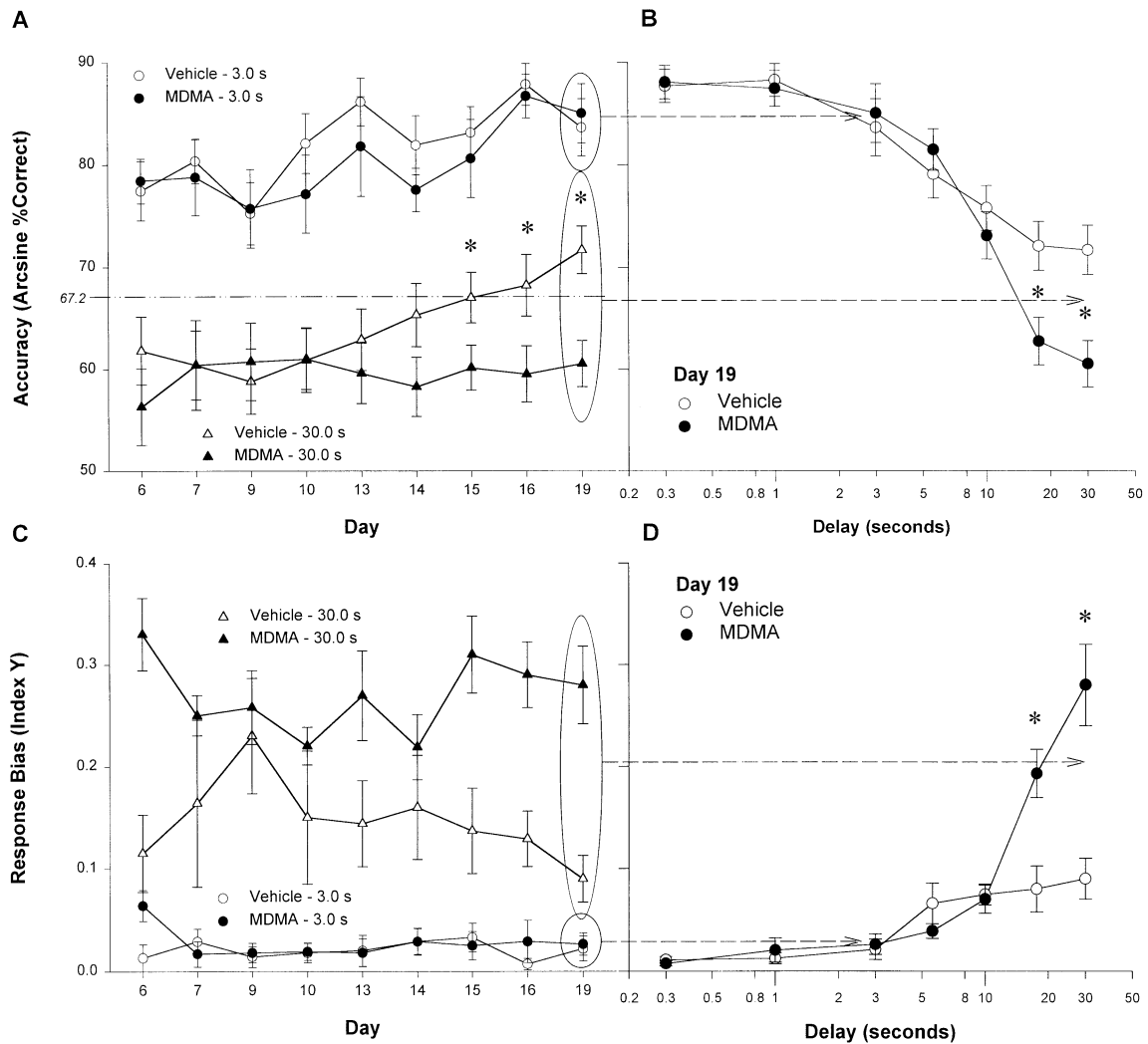


Fig. 4A–D Delayed non-match to place (DNMTP) accuracy and response bias. **A** Accuracy of performance of the DNMTP task as represented by percent scores, following arcsine transformation, for the post-treatment phase (too few trials completed during treatment phase to make analysis meaningful). Data from two of the seven delays are plotted as representative. The *upper lines (circles)* plot the accuracy at the 3-s delay, while the *lower (triangles)* show the 30-s delay data. The *dash and dotted line* plotted at 67.2 arcsine % represents the 85% overall criterion to which the animals were trained. The data from day 19 (**A**) are extrapolated and partially re-plotted (as indicated) in **B**, where the whole delay function is plotted for each treatment group. The data plotted represent mean and SEM for MDMA ($n=9$) and vehicle ($n=9$) treated groups. $*P<0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment. **C** The “cognitive” bias of animals performing the DNMTP task, as represented by Index Y scores, for the post-treatment phase (too few trials completed during treatment phase to make analysis meaningful). Data from two of the seven delays is plotted as representative. The *upper lines (circles)* plot the accuracy at the 3-s delay, while the *lower (triangles)* show the 30-s delay data. The data from day 19 (**C**) are extrapolated and partially re-plotted (as indicated) in **D**, where the whole delay function is plotted for each treatment group. The data plotted represent mean and SEM for MDMA ($n=9$) and vehicle ($n=9$) treated groups. $*P<0.05$ as determined by analysis of variance with post hoc comparison to the vehicle treatment

on each of the drug treatment days ($P<0.01$). Across the 3 drug treatment days, there was a trend towards a smaller impairment accompanied by a reduction in variance, though this failed to reach significance.

Delayed non-match to position

Inspection of the Trials data and the beam break rate (mean breaks per second of the five longest programmed delays) revealed a disruption of performance by MDMA only during the drug administration phase (Fig. 3). ANOVA confirmed significant main effects of both Session and Treatment, but not Delay for beam breaks. In addition, there were significant interaction terms between Session and Treatment for both Trials [$F_{(11,168)}=5.84$, $P<0.005$] and Beam breaks [$F_{(11,840)}=11.26$, $P<0.001$]. Post hoc analysis demonstrated that the MDMA group performed fewer trials in session 1 ($P<0.01$), while beam break rate was suppressed during the first 2 treatment days ($P<0.01$). MDMA acutely depressed the number of trials completed sufficiently to invalidate any analysis of Accuracy and Bias (Iy) (Sahgal 1987) during the drug treatment period. Thus, investigation of these parameters

was limited to data from the post-treatment, or chronic, phase. Analysis of percent correct data revealed significant main effects of Session, Treatment, and Delay. The three-way interaction was not significant [$F_{(18,1288)}=1.41$, $P>0.05$], though the interactions of Treatment by Delay [$F_{(6,882)}=3.31$, $P<0.005$] and Treatment by Session [$F_{(8,882)}=1.93$, $P<0.05$] were significant. Post hoc analysis of the Treatment by Delay term established a significant difference in accuracy, as measured by percent correct, at the two longest delays, 17.6 and 30 s ($P<0.01$), between the Vehicle and MDMA groups. Further analysis of the Treatment by Session effect revealed a gradual increase in overall accuracy as the experiment proceeded. Performance over the last three sessions was significantly better than the two sessions following cessation of MDMA treatment. Inspection of the means suggested that in general performance was rising across sessions. However, this was not the case for the MDMA group at the longest delays (Fig. 4A). As the two-way interaction may have been masking a smaller, opposite three-way interaction, two restricted analyses were carried out on the 3- and 30-s delay data, respectively, and the ANOVAs appropriately partitioned. This confirmed that interaction between Treatment and Delay did have a significant temporal component in the 30- [$F_{(8,125)}=2.16$, $P<0.05$] but not the 3-s delay data. This effect was attributable to significantly higher levels of accuracy achieved by the Vehicle, with respect to the MDMA treated group on the last 3 days of testing. Further confirmation of the profile of the effect was derived by an analysis restricted to the data from the last test day (Fig. 4B). Post hoc inspection, following a significant Delay by Treatment interaction [$F_{(6,98)}=2.36$, $P<0.05$], confirmed that accuracy at the 17.6- and 30-s delays was significantly poorer in the MDMA as opposed to the Vehicle group. Analysis of the cognitive bias term (Index Iy) revealed main effects of Treatment and Delay and a significant interaction [$F_{(6,882)}=3.55$, $P<0.005$], but not of Session (Fig. 4C). Post hoc analysis confirmed that the MDMA treated animals exhibited significant increase in bias at the longest two delays ($P<0.01$), as illustrated by the data plotted for day 19 in Fig. 4D.

Biochemical analysis

Mono-amine analysis

The effects of repeated administration of MDMA on brain monoamine and metabolite levels were investigated at 3 weeks after the period of exposure to MDMA (Fig. 5A, Table 2). MDMA produced significant decreases in 5-HT [$F_{(1,29)}=35.24$, $P<0.001$] and 5-HIAA [$F_{(1,29)}=35.86$, $P<0.001$] content. This was observed in all brain regions examined; cortex, hippocampus and striatum. By contrast, there were no significant changes in the levels of noradrenaline ($P=0.94$), dopamine ($P=0.41$), DOPAC ($P=0.49$) or HVA ($P=0.18$) 3 weeks after MDMA exposure. It should be noted that the levels

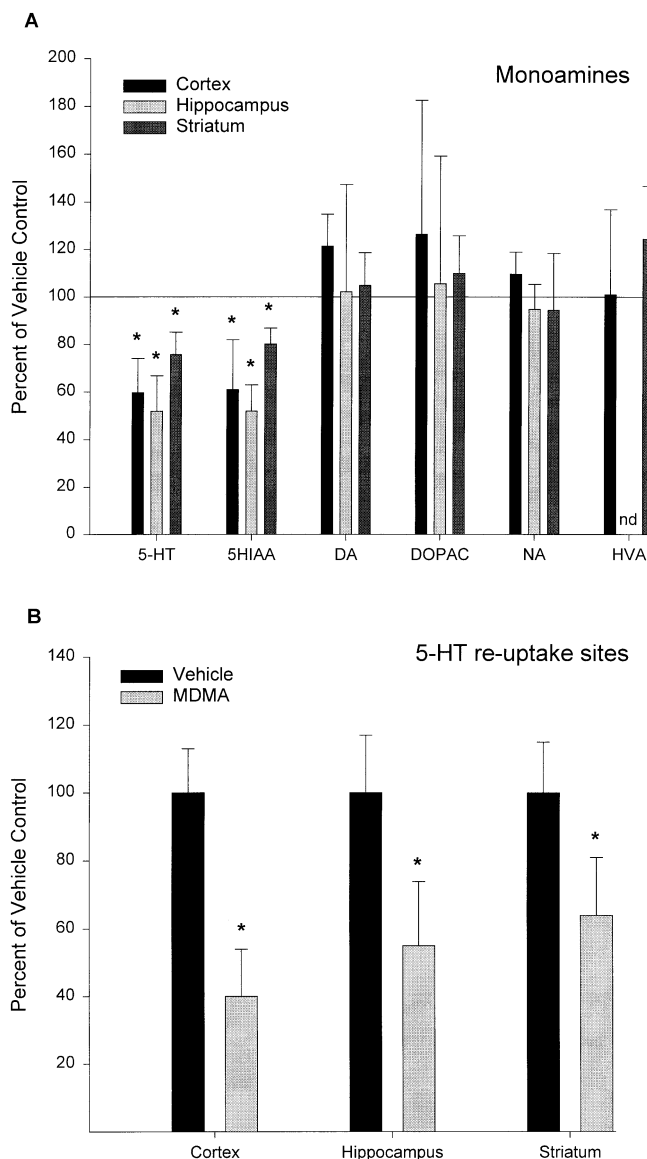


Fig. 5 **A** Monoamine levels in three different brain areas for the control rats ($n=6$) and MDMA treated rats ($n=6$). Each value represents the mean level in tissue from one hemisphere combined for each of the rats in each group expressed as pmol/mg wet weight tissue. Asterisk indicates a significantly different from the control group; $*P<0.05$. **B** Densities of 5-HT re-uptake binding sites determined for three different brain areas from control ($n=6$) and MDMA treated rats ($n=6$). Each value represents the mean density in tissue from one hemisphere combined for each of the rats in each group expressed as percentage of the control value ± 1 SEM. Asterisk indicates a significantly different from the control group, $*P<0.05$

of HVA in the hippocampus were below the level of reliable detection and were therefore not determined.

Citalopram binding

MDMA caused substantial reductions in the density of citalopram binding sites in all brain regions examined

Table 2 Monoamine levels determined by HPLC for three brain areas for control ($n=6$) and MDMA treated rats ($n=6$). Each value represents the mean level in tissue from one hemisphere of each of the rats expressed as pmol/mg wet weight tissue. *Italics* indicate significant difference from control ($P<0.05$). *n.d.* not determined

	5-HT	5 HIAA	NA	DA	DOPAC	HVA
Cortex						
Control	2.14±0.10	1.30±0.07	2.01±0.11	3.69±0.83	0.24±0.06	0.48±0.14
MDMA	<i>1.28±0.13</i>	<i>0.79±0.08</i>	2.21±0.16	4.47±0.49	0.30±0.06	0.48±0.09
Δ%	-40.1	-39.2	+9.9	+21.1	+25.0	0
Hippocampus						
Control	2.39±0.23	2.89±0.19	3.30±0.20	1.30±0.25	0.05±0.02	n.d.
MDMA	<i>1.24±0.27</i>	<i>1.51±0.18</i>	3.12±0.20	1.33±0.27	0.06±0.03	n.d.
Δ%	-48.1	-47.8	-5.5	+2.3	+20.0	-
Striatum						
Control	2.84±0.15	3.43±0.25	0.90±0.17	44.9±3.04	9.70±0.94	3.29±0.29
MDMA	<i>2.13±0.17</i>	2.73±0.21	0.85±0.20	47.1±3.00	10.6±1.33	3.90±0.45
Δ%	-25.0	-20.4	-5.6	+4.9	+9.2	+18.5

Table 3 B_{\max} (pmol/mg protein) and K_D (nM) values shown for [3 H] citalopram binding determined for three different brain areas from control ($n=6$) and MDMA treated rats ($n=6$). Data represent

mean±1 SEM, derived from analysis of one hemisphere of each animal. *Italics* indicate significant difference from control ($P<0.05$)

	B_{\max}			K_D	
	Control	MDMA	Δ%	Control	MDMA
Cortex	3.12±0.31	<i>0.99±0.10</i>	-68.2	1.24±0.12	1.04±0.07
Hippocampus	2.11±0.34	<i>0.81±0.09</i>	-61.6	1.43±0.19	1.05±0.32
Striatum	5.24±0.66	2.76±0.58	-47.3	1.74±0.21	1.66±0.46

(Fig. 5B, Table 3). Analysis confirmed significant main effects of Treatment [$F_{(1,30)}=32.93$, $P<0.001$] and Region with no interaction between the two, MDMA exposure causing a 59% reduction, overall, in the number of binding sites as measured by their B_{\max} with no change in the affinity of the receptors (K_D values). Comparison of Hill coefficients (1.25 saline, 1.03 MDMA) showed that citalopram was binding to a single population of binding sites in both the control and MDMA treated animals. There were no significant differences in the K_D between the control and MDMA treated rats ($P>0.05$).

Discussion

The dosing regimen used in this study bears direct comparison with those reported in human studies. For example, McCann and colleagues (1998) report their subjects as using between 150 and 1250 mg per day (i.e. 2.5–20.8 mg/kg for a 60 kg individual). This would be further emphasised if the usual scaling factors of volume to surface area ratios used when comparing species are considered. The patterns of behavioural effects observed in this study were clearly subdivided into two phases. First, an acute syndrome, with a wide spectrum of effects, apparent during the period of MDMA exposure and second, a cognitive impairment that developed over the post-exposure period. Post mortem, analysis at 17 days confirmed that the MDMA treated animals had suffered significant long-term reductions in cortical 5-HT competence.

Acute phase

The general appearance of the treated animals included components of the classic “serotonin syndrome” (Jacobs 1976; Solivier et al. 1978). This included low body posture, abducted hind limbs, salivation and piloerection. The behavioural patterns were consistent with the hypothesis that acute MDMA exposure results in release of presynaptic serotonin and dopamine (Sprague et al. 1998). It should be noted though that few, if any, of the animals exhibited the full repertoire of effects associated with the administration of the 5-HT_{1A} agonist (±)-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) (Solivier et al. 1978). The data therefore suggest that, even in laboratory rats, there is significant heterogeneity in the response of individual, drug naive subjects to an identical exposure to MDMA.

The thermophysiological data clearly show that MDMA induced a hypothermic reaction immediately following administration. This finding is in agreement with recent reports that MDMA can elicit both hypo- and hyperthermic responses (Colado et al. 1995; Dafters and Lynch 1998; Malberg and Seiden 1998). The critical factor determining the direction of the thermic response was the ambient temperature which, when below 20–22°C, elicits a hypothermic response as in this study conducted at 21°C.

All the behavioural tests (locomotor activity, temperature, DNMT, and skilled paw-reaching performance) were significantly disrupted in the acute phase. The hyperkinesia was immediate, amphetamine-like, short lived

and showed a mild sensitisation with repeated administration of MDMA. As such, the effects are qualitatively similar to those reported in other studies (Paulus and Geyer 1992; Dafters 1994, 1995). The return to the pre-drug baseline on the second session following the cessation of drug treatment suggests that the hyperkinesia was not associated with an ongoing neurodegenerative process. The slight delay was possibly attributable to a behavioural conditioning effect to the context of the locomotor activity boxes, rather than to a residual effect of MDMA. Similarly, skilled motor ability as required for the staircase test was impaired. The rapid return and lack of longer-term decline again suggests that the motor control system is perturbed rather than damaged by exposure to MDMA. This conclusion is supported by the observation that, against the increasing dose administered across the 3 days, the trend in the behavioural data was for a reduction in impairment. This in turn suggests either a behavioural, and/or pharmacological, tolerance to MDMA develops during exposure.

The results of the operant DNMTTP showed that all aspects of performance were severely impaired during the early stages of the acute phase of MDMA exposure. This cannot be interpreted as a specific disruption of cognitive function, in the light of the major perturbations observed in all the other tests. It should be noted that the number of trials completed and the beam break rate returned towards vehicle levels over the acute phase. During this period, the level of hyperkinesia remained constant as the dose of MDMA was increased from 10 to 20 mg/kg per treatment. The attenuation of the effects of repeated MDMA exposure on performance of a complex cognitive task and the observed hypothermia, but not of the spontaneous hyperkinesia and impaired skilled paw reaching, suggests a possible dissociation of effects. This pattern could be explained by a direct effect upon sub-cortical monoaminergic systems mediating the changes in motor output. The impaired DNMTTP performance and hypothermia could have resulted from a stress response to aversive effects of MDMA administration.

Post-treatment phase

Over the 16 days following MDMA treatment, there were no differences between the vehicle and drug treated groups with respect to locomotor activity, paw-reaching ability or body temperature. Further, the level of performance, in terms of trials completed and beam-break rate in the DNMTTP task, was indistinguishable between the two groups. However, it was found that the MDMA treated group did not exhibit the progressive improvement in performance, at the longer delays, seen in the control group at the longer delays. The response strategy of MDMA animals was also significantly more biased than in the controls. The improvement in overall performance in the control group must be attributed to further learning from a sub-asymptotic baseline at the beginning of the experiment. A proportion of the impairment, seen

in the MDMA group, could be attributed to residual effects of the drug treatment or related to an increased stress response. However, the effects only became significant 12 days after the treatment ceased. This would thus forcefully suggest that the various procedural and treatment related perturbations, that could be viewed as stressors, had little impact on performance of the task. Similarly, impairments in DNMTTP performance can be attributed to postural habits leading to mediating strategies. The operant boxes used in this study utilise an infrared detection system to count magazine entries, rather than a Perspex door. Consequently the intra-delay magazine response rate is relatively high (>2 Hz, Fig. 3A) which makes the adoption of position habits much more difficult to sustain. Delay-dependent impairments in accuracy are often attributed to perturbations in short-term memory and related systems. This hypothesis is also supported by the reciprocal trend for a decrease in the bias index, I_y , which became evident at longer delays in the control data. A decrease in bias of this type suggesting that the control animals were developing improved response strategies for the long delay trials. The ability to develop this type of strategy is dependent upon an effective short-term memory system. In recent human studies, MDMA users have been shown to have specific impairments in word recall, a short-term memory task, when tested 7 days after MDMA use (Parrot and Lasky 1998). However, it should be noted that both these deficits might also be mediated, either wholly or in part, by impairments in associated processes such as attentional or central executive, functions.

The post mortem analysis of the brain tissue replicated previous findings in animals of a characteristic pattern of damage indicative of neurotoxic sequelae following MDMA exposure. Depletions of 5-HT and its metabolite 5-HIAA (Schmidt et al. 1986), together with a decrease in the density of 5-HT uptake sites (Battaglia et al. 1987) in cortex, hippocampus and striatum were confirmed. While there were no changes in markers of dopaminergic, or noradrenergic, function. This pattern would support the hypothesis that MDMA is selectively toxic to 5-HT terminal fields. Though a direct causal link cannot be proven, because of the correlational nature of the association, the data suggest that the behavioural effects observed should be attributed specifically to primarily to 5-HT nerve-terminal dysfunction. Further, we can confirm that the reductions in 5-HT indices persist to a point 20 days post-MDMA exposure. This corresponds with the recent findings from *in vivo* PET studies that the density of 5-HT transporters in the neocortex is reduced in baboons for up to 13 months post-MDMA exposure (Scheffel et al. 1998). In humans, widespread reductions in 5-HT uptake sites are present, at least 3 weeks following MDMA use (McCann et al. 1998).

As far as we are aware, no other study has described a long-term syndrome in animals previously treated with MDMA. Ricaurte et al. (1993) reported a failure to observe an impairment in short-term memory following chronic MDMA exposure using a T-maze alternation

test. Closer examination of the results and task involved reveal that the accuracy of the performance of the MDMA treated rats was in fact lower than that of the saline controls, was not statistically significant. Further, the percentage reduction of post mortem 5-HT levels was less than in the present study. Thus we would suggest, the level of task difficulty, the relatively more spatial nature of the T-maze task, and the smaller lesion size, all contributed to the failure of Ricaurte's 1993 study to detect a lasting change. In non-human primate studies similar consequences of acute administration of MDMA on cognitive function were similar to those reported in this study (Frederick et al. 1995a). The long-term effects in primates are less clear. Frederick et al. (1995b) report that using an operant test battery, it was possible to detect tolerance to the deleterious effects of MDMA while the baseline performance was not significantly altered. Nevertheless, post mortem analysis of the brain tissue also failed to reveal any consistent pattern of neurochemical damage. Nonetheless, reliable neurochemical depletions (Ricaurte et al. 1992) and reduced 5-HT immunoreactivity (Fischer et al. 1995) have been reported in other non-behavioural studies. It should therefore be noted that Frederick et al. (1995b) did not rule out the possibility of a link between MDMA exposure and long-term cognitive changes in their conclusion. The pattern of disruption to the 5-HT systems observed in the studies by Ricaurte et al. (1992) and Fischer et al. (1995) suggests that damage to frontal 5-HT systems may underlie the cognitive sequelae reported here. Further, agents that alter 5-HT transmission have been shown to influence mnemonic processes (Vanderwolf 1987; Altman and Norville 1988). However, it should be noted that the effects of MDMA on cognition might not be mediated solely by damage to 5-HT pathways. The neural circuitry of learning and memory is complex and involves numerous brain structures including the hippocampus, cerebral cortex, and thalamus. As all of the above are sensitive to the neurotoxic effects of MDMA (Battaglia et al. 1987; Schmidt 1987; Ricaurte et al. 1992; Fischer et al. 1995), the possibility of complex interactions leading to subtle, or long-term, consequences cannot be ignored.

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