

Distinct periods of developmental sensitivity to the effects of 3,4-($\pm$)-methylenedioxymethamphetamine (MDMA) on behaviour and monoamines in rats

Matthew R. Skelton, Devon L. Graham, Tori L. Schaefer, Curtis E. Grace, Amanda A. Braun, Lindsey N. Burns, Robyn M. Amos-Kroohs, Michael T. Williams and Charles V. Vorhees*

Division of Neurology, Cincinnati Children's Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Abstract

Previous findings showed allocentric and egocentric learning deficits in rats after MDMA treatment from postnatal days (PD) 11–20 but not after treatment from PD 1–10. Shorter treatment periods (PD 1–5, 6–10, 11–15, or 16–20) resulted in allocentric learning deficits averaged across intervals but not for any interval individually and no egocentric learning deficits individually or collectively. Whether this difference was attributable to treatment length or age at the start of treatment was unclear. In the present experiment rat litters were treated on PD 1–10, 6–15, or 11–20 with 0, 10, or 15 mg/kg MDMA q.i.d. at 2-h intervals. Two male/female pairs/litter received each treatment. One pair/litter received acoustic startle with prepulse inhibition, straight channel swimming, Cincinnati water maze (CWM), and conditioned fear in a latent inhibition paradigm. The other pair/litter received locomotor activity, straight channel swimming, Morris water maze (MWM), and locomotor activity retest with MK-801 challenge. MDMA impaired CWM learning following PD 6–15 or 11–20 exposure. In MWM acquisition, all MDMA-treated groups showed impairment. During reversal and shift, the PD 6–15 and PD 11–20 MDMA-treated groups were significantly impaired. Reductions in locomotor activity were most evident after PD 6–15 treatment while increases in acoustic startle were most evident after PD 1–10 treatment. After MK-801 challenge, MDMA-treated offspring showed less locomotion compared to controls. Region-specific changes in brain monoamines were also observed but were not significantly correlated with behavioural changes. The results show that PD 11–20 exposure to MDMA caused the largest long-term cognitive deficits followed by PD 6–15 exposure with PD 1–10 exposure least affected. Other effects, such as those upon MK-801-stimulated locomotion showed greatest effects after PD 1–10 MDMA exposure. Hence, each effect has a different window of developmental susceptibility.

Received 1 March 2011; Revised 16 May 2011; Accepted 19 May 2011; First published online 28 June 2011

Key words: Cincinnati water maze, critical periods, learning and memory, MDMA, Morris water maze.

Introduction

The abuse of 3,4-methylenedioxymethamphetamine (MDMA) is an ongoing issue especially in industrialized countries. As with many other drugs of abuse, there have been reports of MDMA abuse

during pregnancy (Ho *et al.* 2001; Moore *et al.* 2010). While the effects of MDMA on the adult brain have been extensively examined, effects on the developing brain remain poorly understood. It has been shown that developmental MDMA exposure has long-term effects on brain development, but many of the studies do not share a common model to facilitate across-study comparisons (reviewed in Skelton *et al.* 2008). We developed a rat model of late second- and third-trimester human brain development to examine the effects of MDMA and related drugs (Broening *et al.* 2001; Vorhees *et al.* 2000). The development of the rat

* Address for correspondence: C. V. Vorhees, Ph.D., Division of Neurology, Cincinnati Children's Research Foundation, 3333 Burnet Ave, ML 7044, Cincinnati, OH 45229, USA.
Tel.: (513) 636-8622 Fax: (513) 636-3912
Email: charles.vorhees@cchmc.org [C.V.V.]
Email: michael.williams@cchmc.org [M.T.W.]

from postnatal days (PD) 1–20 has been shown to be analogous to late second- and third-trimester human brain development (Clancy *et al.* 2007*a,b*). Specifically, structures involved in learning and memory, such as the hippocampus, are developing during this time (Clancy *et al.* 2007*b*; Rice & Barone, 2000).

Previous studies have shown that MDMA exposure to the neonatal rat leads to learning and memory deficits. In an initial experiment, rats were exposed from PD 1–10 or PD 11–20 (Broening *et al.* 2001). Rats exposed from PD 11–20 showed deficits in route-based egocentric learning and spatial learning and memory, while animals exposed from PD 1–10 did not; indicating that MDMA interferes with later stages of brain development. The MDMA-induced deficits observed from PD 11–20 exposure have been replicated and have been expanded to show that the deficits emerge during adolescence and persist until at least age 1 yr (Cohen *et al.* 2005; Skelton *et al.* 2006, 2009; Vorhees *et al.* 2004; Williams *et al.* 2003). In an attempt to refine the critical period, rats were exposed to MDMA during shorter 5-d intervals (PD 1–5, 6–10, 11–15, or 16–20) (Vorhees *et al.* 2009). MDMA treatment caused trends at all intervals but significant spatial learning deficits were only detected when summed across all four exposure intervals, suggesting that 5-d exposure was not sufficient at any one interval to cause deficits (Vorhees *et al.* 2009). In the same experiment, route-based egocentric learning was not disrupted by any of the 5-d exposure intervals individually or collectively. Taken together with previous studies showing that 10-d exposures caused deficits in both types of learning, these data reveal that length of exposure is a critical factor in MDMA-induced learning and memory deficits.

In order to localize the sensitive period, we treated rats with MDMA during one of three overlapping 10-d intervals: PD 1–10, 6–15, or 11–20. The PD 1–10 exposure was included to test for egocentric learning using a new test paradigm that more specifically assesses route-based learning. When MDMA-treated animals were tested previously in this test [Cincinnati water maze (CWM)] they were tested under various levels of light, which allowed for use of distal cues. To eliminate distal cues herein we tested under infrared lighting (Herring *et al.* 2008). In addition, rats were tested for fear conditioning with a latent inhibition (LI) paradigm in order to assess emotional, fear-based memory in MDMA-treated animals. Acoustic startle response/prepulse inhibition (ASR/PPI) was assessed in order to determine if sensory gating was disrupted in MDMA-treated animals. The PD 11–20 exposure group was included for comparison and because LI

learning had not been previously tested. In addition to examining different periods of MDMA exposure, two doses of MDMA were evaluated (10 and 15 mg/kg) to determine if there were dose–response effects. Finally, this study examined NMDA receptor function by measuring locomotor response to the NMDA receptor agonist MK-801. The NMDA receptor was selected for examination due to its role in spatial learning and memory (Morris, 1989; Morris *et al.* 1986).

Materials and methods

Subjects and treatments

Nulliparous male and female Sprague–Dawley CD (IGS) rats were obtained from Charles River Laboratories (USA). Food (Purina 5006) and filtered water were available *ad libitum*. Litters were culled to 12 pups on PD 1 (birth was designated PD 0) balancing for sex. Litters were randomly assigned to one of three treatment ages (regimens): (1) PD 1–10, (2) PD 6–15, or (3) PD 11–20. Two males and two females per litter were subcutaneously injected four times daily (2 h inter-dose interval) with 0 (saline; Sal), 10 or 15 mg/kg MDMA (MDMA-10 and MDMA-15 respectively; expressed as free base). The 10 mg/kg dose of MDMA (95% pure; NIH, USA) was chosen because it has consistently been shown to induce learning and memory deficits after neonatal treatment (Broening *et al.* 2001; Skelton *et al.* 2006; Vorhees *et al.* 2004, 2007; Williams *et al.* 2003). Dose extrapolation to humans has been discussed previously showing that this regimen produces similar plasma MDMA levels in rats as achieved by humans (Skelton *et al.* 2008). On PD 28, litters were separated from the dam and divided by sex and treatment group and placed in one of two testing arms. Body weights were recorded daily during treatment and weekly thereafter. Twenty litters were used per regimen; hence there were 20 males and 20 females in each treatment/regimen/testing arm. The vivarium is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and protocols were approved by the Institutional Animal Care and Use Committee.

Behavioural testing

Within a litter, one male/female pair/treatment group was assigned to one of two testing arms with the first consisting of ASR/PPI, straight channel swimming, CWM, and conditioned fear/LI. The other male/female pair/treatment group/litter was assigned to: locomotor activity, straight channel swimming, Morris

water maze (MWM), and locomotor activity with MK-801 (NMDA antagonist) challenge. Swimming tasks were conducted with room temperature ($21 \pm 1^\circ\text{C}$) water, and testing began on PD 60.

ASR/PPI

ASR/PPI was measured in an SR Lab apparatus (San Diego Instruments, USA). During testing, a 70 dB background noise was provided. The animal was placed in a restraint device connected to a transducer which converts the animal's movement into an electrical readout (mV). Following 5-min acclimation, the trials began. Trials were of three types: no stimulus (NS), startle stimulus (SS), or SS with a prepulse preceding it. Prepulse (PP) intensity levels were: 73, 75, and 80 dB for 20 ms followed 70 ms later (onset to onset) by a SS of 120 dB for 20 ms. Trials were presented in a Latin squares design such that each trial type was given five times as follows: NS, SS, PP73 + SS, PP75 + SS, and PP80 + SS. Each Latin square was repeated twice for a total of 10 trials of each type. Responses were recorded for 100 ms following stimulus onset. The primary measure was maximum startle amplitude (V_{max}) measured in mV of change relative to background movement (V_0).

Straight channel swimming

Animals were tested on a single day (four trials) for swimming in a straight water channel. Rats were placed in the channel (15 cm wide $\times$ 244 cm long) facing the wall at the opposite end from where a submerged escape platform was located and latency (up to 2 min) was recorded for each trial.

CWM

The CWM has been described previously (Vorhees, 1987) with modification using infrared lighting (Herring *et al.* 2008). Two trials per day were given with a 5-min inter-trial interval on those trials where an animal failed to locate the escape within 5 min. Latency and errors were scored. An error was defined as entry into the stem of a 'T' or into an arm of a T and defined by whether the head and shoulders moved past the line of entry into either a stem or a T. Perseverative errors within a T were counted separately. Animals were tested for 18 d.

Conditioned fear/LI

The day following CWM, animals were placed in conditioned fear chambers (Coulbourn Instruments, USA). Rats in each group were subdivided in two conditions: (a) tone pre-exposed (PE) and (b) tone

non-pre-exposed (NPE). PE animals were placed in the chamber and received 30 tones (82 dB, 2 kHz, 30-s duration) separated by 30-s intervals. The NPE animals received no tone during the same time period. Following the 15-min PE or NPE interval all animals were given three tone-footshock pairings (180 s between pairings) of 0.3 mA lasting 1 s each. Contextual conditioning was assessed 24 h after training. Rats were returned to the chamber with the same floor as during training for 6 min and freezing recorded. 24 h later, rats were placed in the chambers with a different floor (grid rather than bars). Rats were assessed for 3 min with no-tone followed by 3 min with tone. Freezeframe software (Coulbourn Instruments) was used to determine the percentage of time freezing. LI is determined using the treatment $\times$ exposure interaction term of the corresponding analysis of variance (ANOVA).

Locomotor activity and activity following MK-801

Animals were tested for locomotor activity for 1 h in an automated monitor (Accuscan Instruments, USA) as described previously (Skelton *et al.* 2009). For the MK-801 challenge, animals were re-tested in the same apparatus. For the retest, rats were placed in the apparatus for 30 min, in order to re-acclimate the animals to the chamber and limit the effect of initial changes of activity on the MK-801-induced hyperactivity, followed by a s.c. injection of MK-801 (0.2 mg/kg) and 3 h of additional testing.

MWM

On PD 62 testing in the MWM began. Testing was divided in three phases (7 d/phase) consisting of four trials per day (2-min maximum trial; 15-s inter-trial interval) for 6 d to learn the location of the hidden platform followed by a probe trial on day 7 with the platform removed (Vorhees & Williams, 2006). The phases were: acquisition (10 cm platform in the SW quadrant), reversal (7 cm platform in the NE quadrant), and shift (5 cm platform in the NW quadrant). Video tracking software (Smart Track[®], San Diego Instruments, USA) was used to record latency, path length, cumulative distance, and swim speed during hidden platform trials and swim speed and average distance from the platform on probe trials. In addition, performance on the first trial of days 2–6 were examined as tests of memory across days. In order to determine if MDMA learning deficits were due to spatial learning difficulties or represent a global deficit, we separately analysed the performance of animals on day 1 of the acquisition phase. We have shown that treatment with MDMA during the neonatal period

Table 1. Body weight and mortality

Regimen	Treatment	Deceased	Pre-dose weight	Post-dosing weight	Adult weight
PD 1–10	Sal	0/80	6.8 ± 0.3	23.3 ± 0.3	275.4 ± 3.8
	MDMA-10	7/80**	6.8 ± 0.3	19.0 ± 0.3***	259.0 ± 3.9***
	MDMA-15	14/80**	6.8 ± 0.3	18.1 ± 0.3***	255.4 ± 3.9***
PD 6–15	Sal	0/80	14.3 ± 0.4	32.1 ± 0.4	260.9 ± 3.3
	MDMA-10	3/80	14.3 ± 0.4	26.4 ± 0.4***	248.5 ± 3.3***
	MDMA-15	6/80***	14.5 ± 0.4	25.7 ± 0.4***	244.7 ± 3.3***
PD 11–20	Sal	0/80	25.7 ± 0.7	45.0 ± 0.7	267.9 ± 2.8
	MDMA-10	6/80*	25.4 ± 0.7	37.1 ± 0.7***	255.3 ± 2.8***
	MDMA-15	7/78**	25.7 ± 0.7	35.9 ± 0.7***	254.0 ± 2.9***

Pre-dose weight was body weight at commencement of dosing; post-dosing weight was the weight at the end of the dosing period; adult weight is the weight at the beginning of behavioural testing; and deceased is the number dying over the total number treated.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

does not affect performance in the visible platform phase of the MWM (Broening *et al.* 2001; Skelton *et al.* 2009; Vorhees *et al.* 2004; Williams *et al.* 2003), so this task was not repeated.

Neurotransmitters

Following LI testing, animals were decapitated and brains removed and the hippocampus, neostriatum, prefrontal cortex, and entorhinal cortex dissected on ice and stored at -80°C until assayed by HPLC for serotonin (5-HT), norepinephrine (NE) and dopamine (DA) as described previously (Grace *et al.* 2010).

Statistical analysis

The experiment was a split-litter design. Since litter-mates may be regarded as a matching factor (Kirk, 1995), litter was used as a random factor in a completely randomized block ANOVA and each regimen was analysed separately. In this model, litter was the blocking factor with sex and treatment as fixed factors within block. Measures taken repetitively on the same subject were used as a repeated-measures factor. Mixed linear models were used (SAS Proc Mixed version 9.2, SAS Institute Inc., USA). Autoregressive covariance models and Kenward–Roger adjusted degrees of freedom, which can be fractional, were used. Significant interactions were analysed using slice-effect ANOVAs. We have shown previously that developmental MDMA induces learning and memory deficits; therefore, we hypothesized impaired maze learning and hence unidirectional comparisons were appropriate using the step-up Hochberg

method to compare MDMA-treated groups to Sal controls. For locomotor activity following MK-801 challenge, an analysis of covariance (ANCOVA) was performed with the mean of the pre-challenge activity used as the covariate for the post-challenge data. Mortality data were analysed using Fisher's exact test. Pearson product-moment correlation coefficients were calculated for CWM latency and errors averaged across days and hippocampal 5-HT levels. Significance was set at $p \leq 0.05$. Data are presented as least square (LS) means $\pm$ S.E.M.

Results

Body weights and mortality (Table 1)

Regimen PD 1–10

The percentage of progeny that died was 8.8% (7/80) for the MDMA-10 group and 17.5% (14/80) for the MDMA-15 group ($p < 0.01$ vs. Sal). For body weight, main effects of treatment ($F_{2,197} = 87.40$, $p < 0.001$), sex ($F_{1,197} = 6.73$, $p < 0.05$), day ($F_{9,1886} = 2172.87$, $p < 0.001$), and treatment $\times$ day interaction ($F_{18,1898} = 33.11$, $p < 0.001$) were significant. Animals treated with both doses of MDMA showed decreased body weights compared to Sal-treated animals from PD 2–10. In addition, MDMA-15 rats weighed less than MDMA-10 rats from PD 6–10. Females weighed less than males.

Regimen PD 6–15

The percentage of progeny that died was 3.8% (3/80, n.s.) for the MDMA-10 group and 7.5% for the

Table 2. Acoustic startle response/prepulse inhibition and latent inhibition/conditioned fear

Regimen	Treatment	Acoustic startle ($V_{\max}$)				Conditioned fear/latent inhibition (% time freezing)			
		PP0	PP73	PP75	PP80	Day 2 NPE	Day 2 PE	Day 3 NPE	Day 3 PE
PD 1–10	Sal	376.2 ± 39.9	297.6 ± 39.9	241.5 ± 39.9	212.5 ± 39.9	26.4 ± 6.5	14.7 ± 6.4	84.4 ± 7.7	39.7 ± 7.5
	MDMA-10	439.7 ± 40.4	340.0 ± 40.4	311.2 ± 40.4	258.5 ± 40.4	20.1 ± 6.4	7.1 ± 6.9	66.6 ± 7.5	20.6 ± 8.1
	MDMA-15	511.1 ± 41.2	431.3 ± 41.2	386.8 ± 41.2	323.4 ± 41.2	16.8 ± 7.0	12.4 ± 6.4	68.2 ± 8.4	30.0 ± 7.5
PD 6–15	Sal	270.1 ± 20.9	211.1 ± 20.9	165.7 ± 20.9	138.7 ± 20.9	12.1 ± 5.6	19.8 ± 5.6	73.9 ± 8.8	53.7 ± 8.7
	MDMA-10	282.1 ± 21.3	243.7 ± 21.3	197.7 ± 21.3	172.8 ± 21.3	13.6 ± 5.6	14.7 ± 5.9	67.4 ± 8.8	55.9 ± 9.2
	MDMA-15	266.9 ± 21.4	221.5 ± 21.4	194.0 ± 21.4	157.8 ± 21.4	5.0 ± 5.8	21.1 ± 5.7	61.3 ± 9.2	58.0 ± 8.9
PD 11–20	Sal	258.4 ± 25.0	186.8 ± 25.0	158.1 ± 25.1	143.3 ± 25.0	26.4 ± 6.5	14.7 ± 6.4	62.2 ± 9.1	43.2 ± 9.4
	MDMA-10	306.1 ± 25.6	218.4 ± 25.6	188.9 ± 25.6	158.2 ± 25.6	20.1 ± 6.4	7.1 ± 6.9	68.6 ± 9.5	50.9 ± 9.4
	MDMA-15	320.7 ± 26.6	240.3 ± 26.6	201.7 ± 26.6	187.4 ± 26.6	16.8 ± 7.1	12.4 ± 6.4	70.4 ± 9.2	56.0 ± 10.9

PP, Prepulse; PE, pre-exposed; NPE, non-pre-exposed.

Values are least squares mean ± S.E.M.

MDMA-15 group (6/80, $p < 0.01$ vs. Sal). For body weight, main effects of treatment ($F_{2,213} = 142.00$, $p < 0.001$), sex ($F_{1,213} = 16.58$, $p < 0.001$), day ($F_{9,2000} = 1513.32$, $p < 0.001$), and treatment × day interaction ($F_{18,2015} = 41.36$, $p < 0.001$) were significant. Treatment effects were observed from PD 7–15. Both MDMA groups weighed less than the Sal-treated animals from PD 7 throughout dosing. The MDMA-15 group weighed less than the MDMA-10 animals beginning on PD 12. Females weighed less than males.

Regimen PD 11–20

The percentage of progeny that died was 7.5% for the MDMA-10 group (6/80, $p < 0.05$ vs. Sal) and 9% for the MDMA-15 group (7/78, $p < 0.01$ vs. Sal). For body weight, main effects of treatment ($F_{2,210} = 129.84$, $p < 0.001$), sex ($F_{1,210} = 24.59$, $p < 0.001$), day ($F_{9,1909} = 734.62$, $p < 0.001$), and treatment × day interaction ($F_{18,1923} = 32.74$, $p < 0.001$) were significant. Treatment effects were observed from PD 12–20. Both MDMA-treated groups weighed less than Sal-treated animals beginning on PD 12. The MDMA-15 group weighed less than the MDMA-10 group on PD 20 only. Females weighed less than males.

Post-weaning body weight

Weights were taken weekly after PD 28. Significant main effects of treatment ($F_{2,660} = 70.88$, $p < 0.001$), sex ($F_{1,659} = 5634$, $p < 0.001$), and age ($F_{6,3777} = 12197.4$, $p < 0.001$) were observed. In addition, treatment × sex ($F_{2,659} = 10.88$, $p < 0.001$), sex × age ($F_{6,3778} = 1175.21$, $p < 0.001$), and regimen × age ($F_{12,3819} = 7.43$, $p < 0.001$) interactions were observed. Males treated with MDMA, regardless of dose, weighed less than

Sal-treated animals, and MDMA-15 males weighed less than MDMA-10 males. Among females, both MDMA-treated groups weighed less than the Sal-treated group; no differences were observed between the two MDMA-treated groups.

ASR/PPI

For all regimens, main effects of prepulse intensity and sex were observed ($p < 0.05$). Startle reflex was inhibited by the prepulse and males showed larger startle responses compared to females. Values are given in Table 2.

In the PD 1–10 regimen, a main effect of treatment was observed [$F_{2,85.3} = 4.79$, $p < 0.05$; $V_{\max}$ (mV) across prepulse intensities (LS mean ± S.E.M.): Sal = 281.9 ± 38.3 ; MDMA-10 = 337.4 ± 38.8 ; MDMA-15 = 413.1 ± 38.8]. No interaction of treatment × prepulse was obtained. Regardless of prepulse level, MDMA-15 animals had increased startle responses compared to Sal animals ($p < 0.01$) while MDMA-10 animals did not differ from either Sal or MDMA-15 animals.

In the PD 6–15 and PD 11–20 exposure groups, no main effects or interactions with treatment were observed.

Locomotor activity

For the PD 1–10 regimen, there was a main effect of treatment ($F_{2,79.2} = 8.12$, $p < 0.001$, Fig. 1): both MDMA-treated groups showed reduced activity compared to Sal-treated animals ($p < 0.01$).

In the PD 6–15 regimen, there was a main effect of treatment ($F_{2,92.9} = 20.9$, $p < 0.001$) and a treatment × interval interaction ($F_{22,1095} = 1.88$, $p < 0.01$). Both MDMA-treated groups showed reduced activity

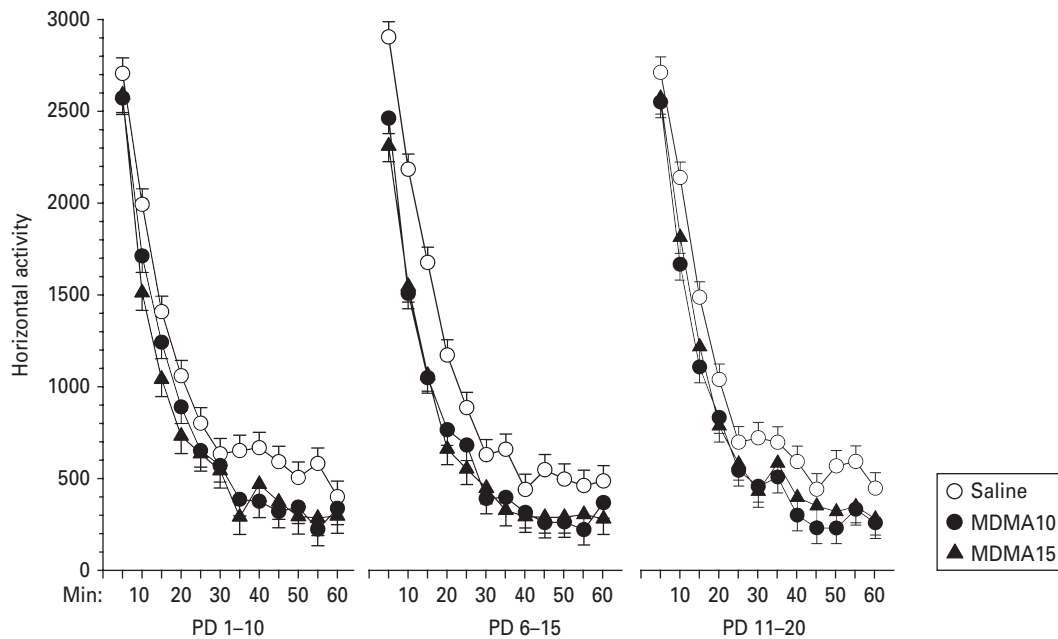


Fig. 1. Developmental MDMA exposure leads to hypoactivity. Locomotor activity was assessed for 1 h in a novel environment. Regardless of dosing regimen, MDMA-treated animals had lower activity levels than Sal-treated animals. Data are presented as least squares mean $\pm$ S.E.M. ($n = 16$ – 20 /group).

compared to Sal animals ($p < 0.001$). MDMA-treated animals were less active than Sal animals during the first 25 min and from 30–35 min and 40–45 min.

In the PD 11–20 regimen there was a main effect of treatment ($F_{2,89.9} = 9.81$, $p < 0.001$). Both MDMA-treated groups showed reduced activity compared to Sal-treated animals ($p < 0.001$).

Straight channel swimming

There were no treatment, sex, or treatment-related interactions for any regimen, nor were there any treatment-related effects in the two subgroup arms of the study that both received straight channel testing.

MWM

Latency, path length, and cumulative distance are highly correlated (Vorhees & Williams, 2006) and showed convergent effects; therefore, path length was used to reflect the findings on platform trials, including the analysis of daily trial-1 data as an index of memory improvement. Average distance was the principal index used on probe trials.

Acquisition

Regimen PD1–10: there was a main effect of treatment ($F_{2,82} = 4.33$, $p < 0.05$) but no treatment-related

interactions. Both MDMA-treated groups had longer paths than Sal-treated animals (Fig. 2a, left). On trial 1, day 1, there were no effects of treatment on path length (LS mean $\pm$ S.E.M.: Sal = 25.7 ± 1.8 ; MDMA-10 = 29.9 ± 1.9 ; MDMA-15 = 30.8 ± 2.1). *Regimen PD 6–15:* there was a main effect of treatment ($F_{2,92.1} = 4.84$, $p < 0.01$) but no treatment-related interactions; both MDMA-treated groups had longer paths than Sal-treated animals (Fig. 2a, middle). On trial 1, day 1, there was no effect of treatment on path length (Sal = 28.1 ± 1.5 ; MDMA-10 = 33.1 ± 1.5 ; MDMA-15 = 31.4 ± 1.5). *Regimen PD 11–20:* there was a main effect of treatment ($F_{2,86} = 8.33$, $p < 0.001$) but no treatment-related interactions; both MDMA-treated groups showed increased paths compared to Sal (Fig. 2a, right). There was no effect of treatment on path length for trial 1, day 1 (Sal = 27.8 ± 1.8 ; MDMA-10 = 31.0 ± 1.8 ; MDMA-15 = 31.8 ± 1.9). There were no significant differences between the MDMA-10 and MDMA-15 groups for any regimen.

In regimen PD 1–10 and PD 6–15, sex main effects were observed with males having shorter path lengths than females. No treatment effects were observed for swim speed.

Probe trials. During the probe trial 24 h after the last learning trial and on trial 1 of days 2–6, no effects of treatment were observed for the PD 1–10 or PD

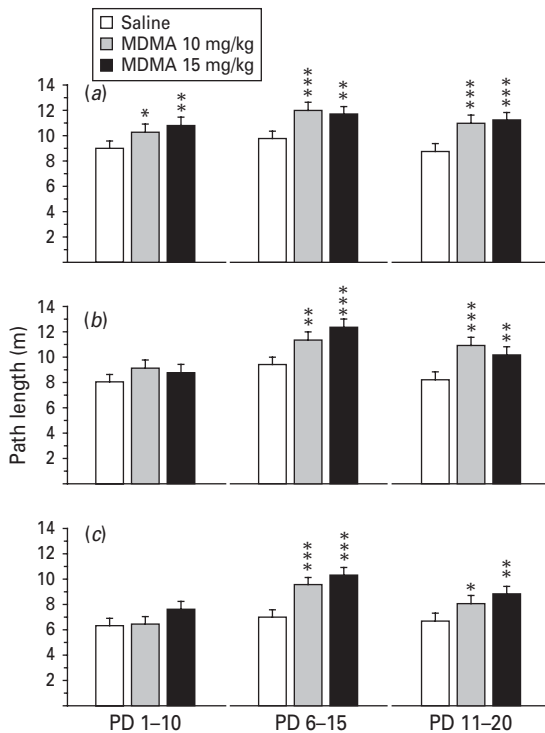


Fig. 2. MDMA induced spatial learning deficits in the Morris water maze (MWM). Rats were tested in the MWM for 6 d (four trials/day) for each phase: acquisition, reversal, and shift. Rats treated with MDMA from either PD 6–15 or PD 11–20 showed increased path lengths in each phase [(a) acquisition, (b) reversal and (c) shift] of the test compared to Sal animals. PD 1–10 MDMA treatment caused increased path lengths only for the acquisition phase compared to Sal treatment. Data are presented as least squares mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 16$ –20/group).

6–15 regimens. For the PD 11–20 regimen animals both MDMA-10 and MDMA-15 groups showed increased average distance from the platform during probe trials 24 h after learning trials compared to Sal animals ($p < 0.05$; Fig. 3, left). On trial 1 of days 2–6, a treatment main effect trend was observed for the PD 11–20 regimen ($F_{2,86.9} = 2.93$, $p < 0.10$); the MDMA-15 group had longer paths compared to Sal ($p < 0.05$) and the MDMA-10 group showed a similar tendency compared to Sal ($p < 0.10$).

Reversal

Regimen PD 1–10: there were no significant treatment main effects or treatment-related interactions for path length (Fig. 2b, left). **Regimen PD 6–15:** there was a main effect of treatment ($F_{2,93.7} = 6.95$, $p < 0.01$) with both MDMA groups having longer paths compared to Sal-treated animals (Fig. 2b, middle); there were no treatment-related interactions. The MDMA-10 and

MDMA-15 groups did not differ from one another. A main effect of swim speed was observed in the PD 6–15 regimen because the MDMA-10 group swam *faster* than Sal-treated animals; no differences in the MDMA-15 group were observed. **Regimen PD 11–20:** there was a main effect of treatment ($F_{2,87.6} = 5.82$, $p < 0.01$); both MDMA-treated groups had longer paths compared to Sal with no differences between the MDMA groups (Fig. 2b, right); there were no treatment-related interactions. No effects of swim speed were observed in the PD 1–10 or PD 11–20 regimens.

Probe trials. No differences were observed for average distance in the PD 1–10 or PD 6–15 regimens (Fig. 3, middle). In the PD 11–20 regimen, a main effect of treatment was observed ($F_{2,85} = 5.32$, $p < 0.01$) with the MDMA-10 group having a longer average distance from the platform than Sal-treated animals (Fig. 3, middle). A trend towards a longer average distance to the platform was observed in the MDMA-15 group compared to Sal animals ($p < 0.10$). On trial 1 of days 2–6, the PD 1–10 and PD 6–15 regimens showed no significant effects or trends. In the PD 11–20 regimen there was a main effect ($F_{2,87.1} = 3.38$, $p < 0.05$) of treatment on path length. The MDMA-10 group had longer paths on the first trial of each day compared to Sal; the MDMA-15 group did not differ from Sal.

Shift

Regimen PD 1–10: there was no treatment main effect ($F_{2,82.6} = 2.78$, $p < 0.07$) and no treatment-related interaction (Fig. 2c, left). **Regimen PD 6–15:** there was a main effect of treatment ($F_{2,92.7} = 9.83$, $p < 0.001$) with both MDMA groups showing increased paths compared to Sal (Fig. 2c, middle); there were no treatment-related interactions. **Regimen PD 11–20:** there was a main effect of treatment ($F_{2,87.4} = 5.53$, $p < 0.01$) but no treatment-related interactions. Both MDMA-treated groups had increased paths compared to Sal-treated animals (Fig. 2c, right). For all regimens, main effects of swim speed were observed. For each regimen the MDMA-10 group swam *faster* than Sal-treated groups.

Probe trials. There were no effects for average distance in the PD 1–10 regimen (Fig. 3, right) or in the PD 6–15 regimen. In the PD 11–20 regimen, there was a main effect of treatment ($F_{2,85} = 4.56$, $p < 0.05$); both MDMA-treated groups were farther on average from the platform site than the Sal-treated group (Fig. 3, right). The MDMA-10 and MDMA-15 groups did not differ from one another. No significant effects were observed during trial 1 of days 2–6 for any regimen.

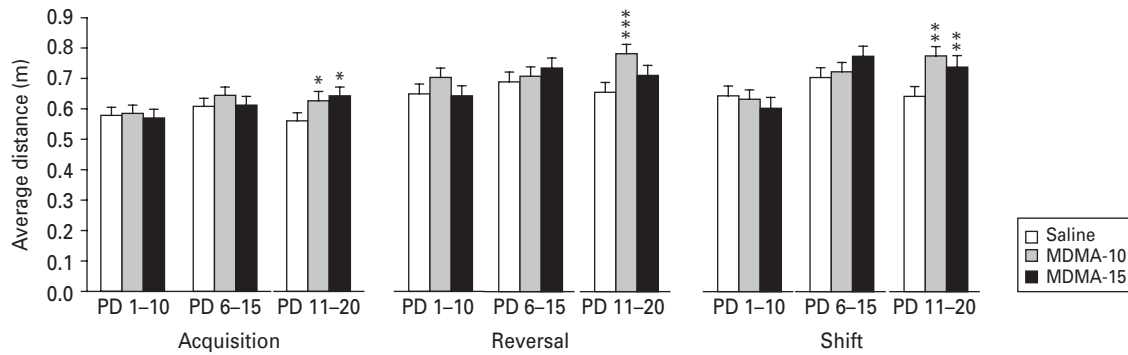


Fig. 3. MDMA induced memory deficits in the Morris water maze (MWM). Probe trials were conducted following each phase of MWM testing. MDMA-treated animals from the PD 11–20 dosing regimen had increased average distance from the platform site compared to Sal. No deficits were observed in the PD 1–10 or PD 6–15 dosing regimens. Data are presented as least squares mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 16$ – 20 /group).

Learning curves. In order to illustrate the rates of learning, path length for each phase of the MWM is shown for each group and regimen in Fig. 4.

Cincinnati water maze

Regimen PD 1–10: there were no treatment effects on latency or errors (Fig. 5). *Regimen PD 6–15:* there were main effects of treatment for latency ($F_{2,86.5} = 6.48$, $p < 0.01$; Fig. 5) and errors ($F_{2,88} = 6.35$, $p < 0.01$); there were no treatment-related interactions. Both MDMA-treated groups committed more errors and had a longer latency than Sal-treated animals ($p < 0.05$). *Regimen PD 11–20:* there was a main effect of treatment for latency ($F_{2,80} = 7.70$; $p < 0.001$) and errors ($F_{2,80.6} = 7.56$, $p < 0.001$). The MDMA-10 and MDMA-15 groups had longer latencies and more errors compared to Sal-treated animals (Fig. 5). In addition, a treatment $\times$ day effect ($F_{28,1173} = 1.52$, $p < 0.05$) was observed. Slice-effect ANOVAs showed significant treatment effects on days 5–12. The MDMA-10 group committed more errors on days 5–7, 9, 11, and 12 and the MDMA-15 group committed more errors on all days compared to the Sal group.

Learning curves: In order to illustrate rates of learning, errors in the CWM are shown for each group and regimen in Fig. 6.

Conditioned fear/LI

No treatment effects were observed in percent freezing during contextual testing for any regimen (Table 2). For cued conditioning, the PD 1–10 regimen showed a main effect of treatment ($F_{2,82} = 3.72$, $p < 0.05$; LS means $\pm$ S.E.M. for percent freezing: Sal = 62.1 ± 5.4 ; MDMA-10 = 43.6 ± 5.5 ; MDMA-15 = 49.1 ± 5.6) and freezing in response to tone ($F_{1,82} = 33.28$, $p < 0.001$).

The MDMA-10 group had reduced freezing compared to Sal-treated animals, while the MDMA-15 group did not differ from Sal animals. Animals in the NPE groups showed more freezing compared to PE groups but the differences between NPE and PE (LI) was not significantly related to treatment. In the PD 6–15 and PD 11–20 regimens, no effects of treatment or tone were observed and no effects on LI were detected.

Locomotor activity following MK-801

Animals were rehabilitated to the locomotor chambers for 30 min prior to MK-801 challenge. No effect of treatment was observed during pre-challenge activity in the PD 1–10 regimen (Fig. 7a). In the PD 6–15 regimen, a main effect of treatment ($F_{2,93.1} = 4.38$, $p < 0.05$; Fig. 7b) was observed during pre-challenge testing. Both MDMA-treated groups showed reduced activity compared to Sal-treated controls ($p < 0.05$). In the PD 11–20 regimen, a main effect of treatment ($F_{2,87.1} = 5.20$, $p < 0.01$) during pre-challenge testing was also seen; and again both MDMA-treated groups had reduced activity compared to Sal ($p < 0.05$). Since MDMA-treated animals showed changes in pre-challenge activity, the mean of the pre-challenge data was used to analyse the post-challenge data by ANCOVA.

Following MK-801, a main effect of treatment ($F_{2,154} = 3.80$, $p < 0.05$) was observed for the PD 1–10 regimen (Fig. 7a). Both the MDMA-15 and MDMA-10 group showed reduced MK-801-induced hyperactivity compared to Sal animals ($p < 0.05$). A treatment $\times$ sex interaction ($F_{2,155} = 3.74$, $p < 0.05$) was also observed. In males, the MDMA-10 group showed decreased MK-801-induced hyperactivity compared to the Sal group ($p < 0.05$), while the MDMA-15 group showed no change compared to Sal. In females, no altered response was seen in the MDMA-10 group while

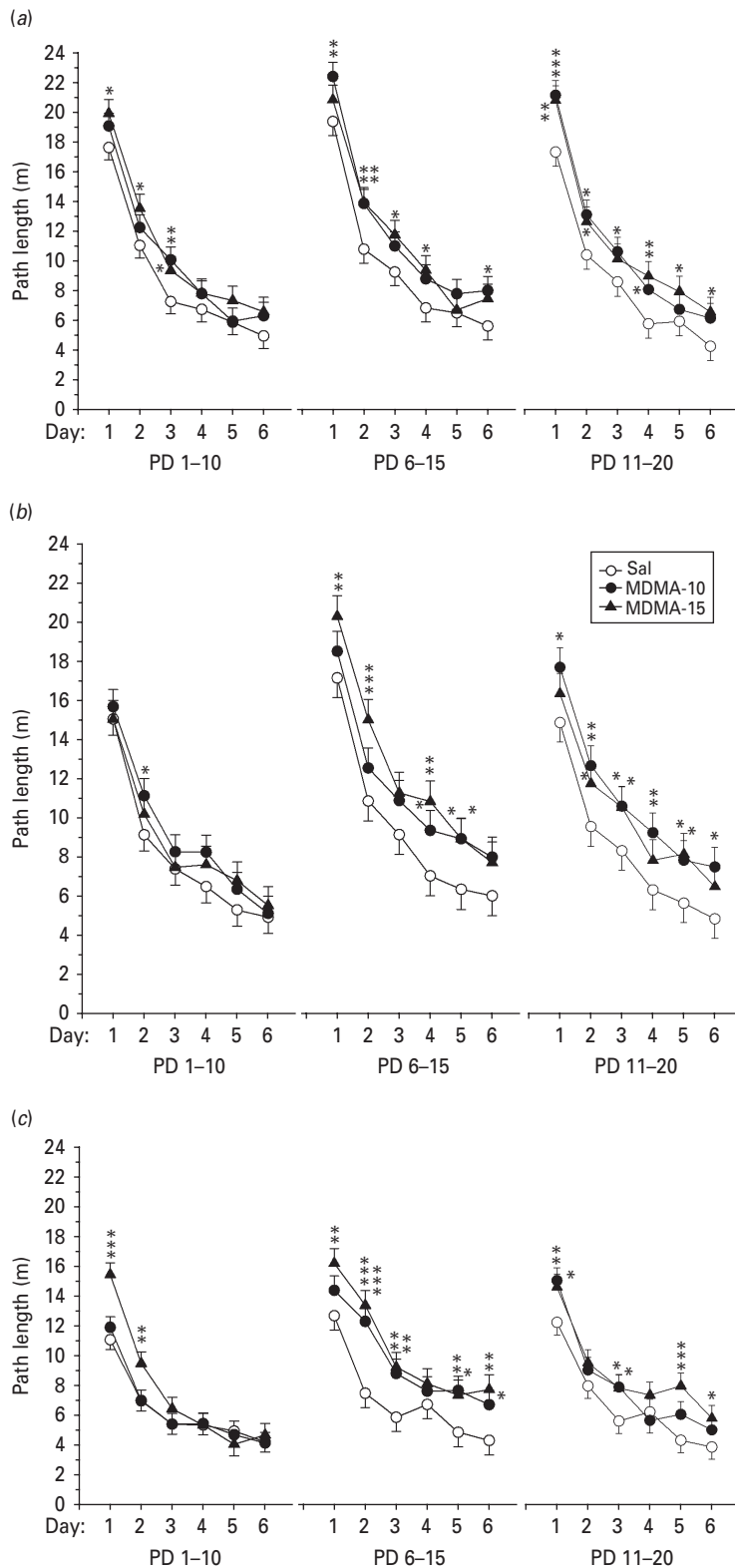


Fig. 4. Morris water maze learning curves. While all animals learned during the (a) acquisition, (b) reversal and (c) shift trials, MDMA-treated animals showed increased path length to the platform compared to Sal-treated animals. Data are presented as least squares mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 16$ –20/group).

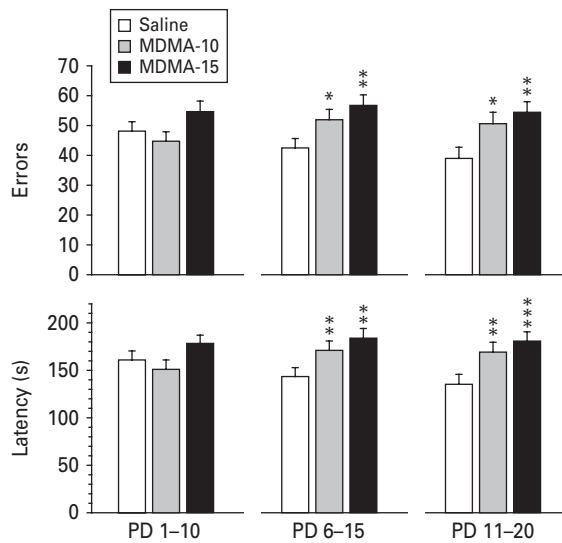


Fig. 5. MDMA-induced route-based learning deficits in the Cincinnati water maze (CWM). Rats were tested in the CWM for 18 d. MDMA-treated animals from the PD 6–15 and PD 11–20 dosing regimens showed increases in latency and errors compared to Sal-treated animals. Data are presented as least squares mean $\pm$ S.E.M. of data averaged across days. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 17$ – 20 /group).

the MDMA-15 group showed lower MK-801-induced hyperactivity compared to the Sal group ($p < 0.001$). In addition, females treated with Sal showed a decrease in MK-801-induced locomotor activity compared to males treated with Sal ($p < 0.05$).

In the PD 6–15 regimen, there was a main effect of treatment ($F_{2,109} = 8.43$, $p < 0.001$) post-challenge (Fig. 7b). Decreased MK-801-induced hyperactivity was observed in both MDMA-treated groups compared to Sal animals ($p < 0.01$). Additionally, there was a treatment $\times$ interval interaction ($F_{34,1721} = 2.28$, $p < 0.001$) with treatment effects observed for intervals 2–8 and 12–18 (p 's < 0.05). Pairwise comparisons showed that both MDMA-treated groups had reduced levels of hyperactivity during intervals 2–8 compared to the Sal-treated group, while the MDMA-10 group had reduced hyperactivity compared to the Sal group during intervals 12–18 ($p < 0.05$). The MDMA-15 group showed a trend ($p < 0.10$) for reduced hyperactivity compared to the Sal group during intervals 12–14. In addition, the MDMA-10 group had reduced hyperactivity compared to the MDMA-15 group during intervals 15–18 ($p < 0.05$).

In the PD 11–20 regimen, there was no main effect of treatment on post-challenge locomotor activity, nor were there any interactions of treatment with other factors (Fig. 7c).

Neurotransmitters

In the entorhinal cortex, no effect was seen in neurotransmitter levels.

In the prefrontal cortex, a main effect of treatment was observed for 5-HT ($F_{2,41} = 3.77$, $p < 0.05$; Table 3) in the PD 11–20 regimen. Pairwise comparisons showed decreased 5-HT in the MDMA-10 group compared to the Sal group ($p < 0.05$). No differences were observed in either the PD 1–10 or PD 6–15 regimens. No treatment effects were observed in DA or NE levels in the prefrontal cortex.

In the neostriatum, there was a main effect of treatment ($F_{2,41} = 3.54$, $p < 0.05$) for DA in the PD 11–20 regimen. The MDMA-15 group had lower DA levels compared to the Sal group ($p < 0.05$), while MDMA-10 did not differ from control. No differences were observed in the PD 1–10 or the PD 6–15 regimen for DA. For 5-HT in the PD 1–10 regimen, a main effect of treatment ($F_{2,38} = 3.06$, $p < 0.05$) was observed with the MDMA-10 animals having higher 5-HT levels compared to Sal animals ($p < 0.05$). In the PD 11–20 regimen, a main effect of treatment ($F_{2,40} = 3.28$, $p < 0.05$) was observed for 5-HT levels with the MDMA-15 group having higher levels in the neostriatum compared to both Sal and MDMA-10 groups (p 's < 0.05). No differences in 5-HT levels were found in the PD 6–15 regimen in the neostriatum.

In the hippocampus, no difference was seen in NE levels for the PD 1–10 regimen. In the PD 6–15 regimen a main effect of treatment ($F_{2,54} = 3.08$, $p < 0.05$) was observed with the MDMA-15 group having higher NE levels compared to the Sal group ($p < 0.05$). No differences in NE levels were observed in the PD 11–20 regimen. For 5-HT, a main effect of treatment ($F_{2,40} = 3.58$, $p < 0.05$) was observed in the PD 1–10 regimen with the MDMA-10 group showing lower 5-HT ($p < 0.05$) levels compared to the Sal group. No differences were observed in the MDMA-15 group compared to the Sal group. No difference was observed in the PD 6–15 or PD 11–20 regimens in hippocampal 5-HT. Correlation coefficients were calculated between the CWM and hippocampal 5-HT levels. No significant correlation was observed between 5-HT and latency ($r = -0.12$, n.s.) or 5-HT and errors ($r = -0.12$, n.s.).

Discussion

The results show that PD 6–15 and PD 11–20 are both sensitive periods for MDMA-induced learning and memory deficits. Animals in both exposure groups showed MWM spatial and CWM route-based learning deficits. MDMA-treated animals showed a 30–40% increase in path length relative to Sal-treated animals,

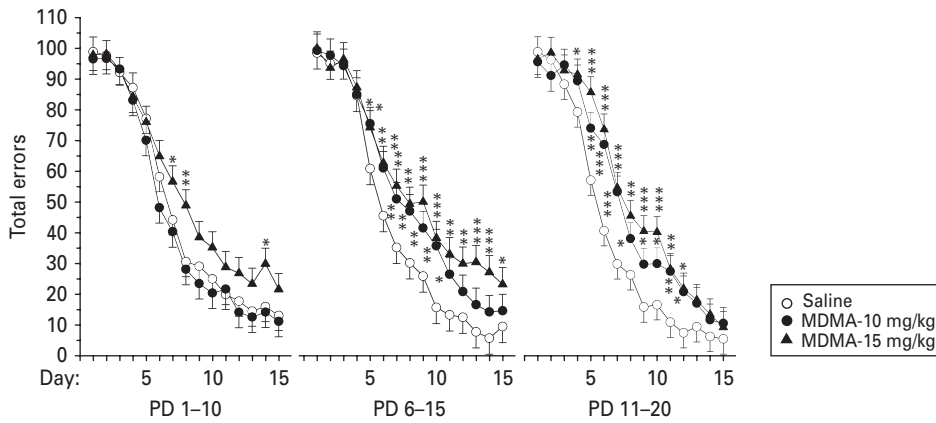


Fig. 6. Cincinatti water maze learning curves for errors committed. Errors committed by all animals decreased as testing progressed; however, MDMA-treated animals commit more errors than Sal-treated animals throughout testing. Data are presented as least squares mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 17$ – 20 /group).

suggesting a significant impairment in spatial learning. Interestingly, fear-based memory was not affected by MDMA administration, suggesting that MDMA exposure does not cause global memory deficits. The increases in path length during the acquisition phase, but not during the later phases, in the PD 1–10 group could be indicative of a less severe spatial learning deficit compared to the other treatment regimens. It is also possible that MDMA-treated animals do not have a spatial learning deficit *per se*. Future studies should be designed to determine if there are subordinate skills that partially contribute to the spatial deficits seen during the acquisition phase of the MWM. This would include performing a pretraining phase in half of an MDMA-treated group compared to a non-pretrained MDMA-exposed group as has been done previously in experiments using NMDA receptor antagonists and methamphetamine administration during development (Cain, 1997; Saucier *et al.* 1996; Williams *et al.* 2002).

In terms of reference memory, in addition to the probe trial given 24 h after the last training trial, we analysed trial 1 on days 2–6 of each phase to obtain additional data on memory improvement across days. We reasoned that trial 1 at the start of each day of testing requires memory of the location of the platform learned the previous day. No reference memory effects were observed in the PD 1–10 or PD 6–15 regimens on these trials or on probe trials given 24 h after the last platform trial. Memory deficits in the PD 11–20 regimen were seen on both daily first trials and on probe trials, a finding consistent with previous probe trial findings (Broening *et al.* 2001; Skelton *et al.* 2006, 2008, 2009; Vorhees *et al.* 2004; Williams *et al.* 2003). It has been shown that the MDMA-induced learning deficits

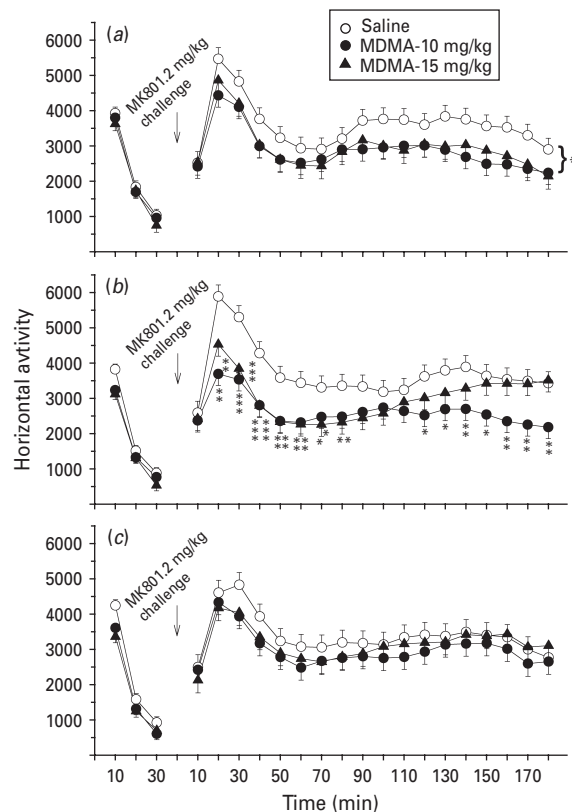


Fig. 7. Blunted MK-801-induced locomotor activation in MDMA-treated rats. Rats were habituated to the locomotor chamber for 30 min followed by a 0.1 mg/kg MK-801 challenge and tested for 180 min thereafter. MDMA-treated rats from the (a) PD 1–10 and (b) PD 6–15 dosing regimens show a blunted hyperactivity after MK-801 treatment compared to Sal-treated animals. (c) The PD 11–20 dosing regimen did not show an altered response to MK-801. Data are presented as least squares mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 17$ – 20 /group).

Table 3. Neurotransmitter levels

Regimen	Treatment	Entorhinal cortex			Prefrontal cortex			Neostriatum		Hippocampus	
		DA	NE	5-HT	DA	NE	5-HT	DA	5-HT	NE	5-HT
PD 1–10	Sal	0.09 ± 0.02	0.38 ± 0.03	0.41 ± 0.04	0.06 ± 0.01	0.31 ± 0.05	0.45 ± 0.07	5.3 ± 0.7	0.22 ± 0.05	0.31 ± 0.03	0.26 ± 0.02
	MDMA-10	0.05 ± 0.02	0.35 ± 0.03	0.30 ± 0.04	0.08 ± 0.01	0.39 ± 0.05	0.49 ± 0.07	6.0 ± 0.7	0.28 ± 0.05*	0.32 ± 0.03	0.21 ± 0.02*
	MDMA-15	0.06 ± 0.04	0.36 ± 0.04	0.35 ± 0.04	0.07 ± 0.01	0.36 ± 0.05	0.46 ± 0.07	5.7 ± 0.8	0.27 ± 0.05	0.37 ± 0.03†	0.25 ± 0.02
PD 6–15	Sal	0.05 ± 0.01	0.33 ± 0.04	0.42 ± 0.03	0.06 ± 0.01	0.33 ± 0.04	0.42 ± 0.04	6.6 ± 0.7	0.30 ± 0.04	0.32 ± 0.02	0.27 ± 0.02
	MDMA-10	0.06 ± 0.01	0.36 ± 0.02	0.37 ± 0.03	0.06 ± 0.01	0.36 ± 0.04	0.42 ± 0.04	6.0 ± 0.7	0.36 ± 0.04	0.35 ± 0.02	0.22 ± 0.03
	MDMA-15	0.06 ± 0.01	0.37 ± 0.02	0.39 ± 0.03	0.05 ± 0.01	0.29 ± 0.04	0.37 ± 0.04	7.0 ± 0.7	0.31 ± 0.04	0.37 ± 0.02*	0.22 ± 0.03
PD 11–20	Sal	0.06 ± 0.02	0.45 ± 0.10	0.54 ± 0.11	0.07 ± 0.01	0.32 ± 0.03	0.46 ± 0.05	8.7 ± 0.9	0.34 ± 0.06	0.35 ± 0.04	0.25 ± 0.02
	MDMA-10	0.07 ± 0.02	0.42 ± 0.11	0.42 ± 0.11	0.04 ± 0.01	0.29 ± 0.04	0.33 ± 0.05**	7.7 ± 0.9	0.32 ± 0.07	0.37 ± 0.02	0.24 ± 0.02
	MDMA-15	0.07 ± 0.02	0.43 ± 0.11	0.46 ± 0.11	0.07 ± 0.01	0.32 ± 0.03	0.42 ± 0.05	6.8 ± 0.9**	0.44 ± 0.07*	0.34 ± 0.04	0.22 ± 0.02

Values are $\mu\text{g/g}$ tissue weight (least squares mean $\pm$ S.E.M.).

* $p < 0.05$, ** $p < 0.01$.

are not due to malnutrition by using large litter controls to match the body weight reductions seen in MDMA-treated offspring; nor are MDMA-induced learning deficits the result of injection stress because the inclusion of untreated controls showed no differences compared to Sal-injected controls (Williams *et al.* 2003). There was some toxicity from MDMA treatment but these effects were largely transient. Moreover, there are no differences in swim performance in the straight channel, suggesting that animals do not have underlying performance deficits. Additionally, we have shown that MDMA-treated animals do not have deficits in the visible platform performance in the MWM (Broening *et al.* 2001; Skelton *et al.* 2006; Vorhees *et al.* 2007, 2009; Williams *et al.* 2003), suggesting that MDMA exposure does not cause sensorimotor deficits. The swim speed effect seen in PD 6–15 animals would not be expected to impair performance since MDMA animals swam faster than controls; nor were similar differences observed on other phases of the MWM or in previous studies with this drug. The lack of acoustic startle deficits supports the hypothesis that MDMA does not cause sensory deficits. Together, these data suggest that there are different, overlapping sensitive periods of MDMA exposure that impair distinct types of navigation and that the sensitive period is greatest from PD 11–20, less from PD 6–15, and much less from PD 1–10.

The changes observed in 5-HT, DA, and NE did not correlate with the learning and memory deficits caused by MDMA. For example, 5-HT changes were seen in PD 1–10 animals that showed only minor learning and memory effects. Serotonin has been implicated in brain development (Sodhi & Sanders-Bush, 2004) and 5-HT release in the developing brain could

lead to aberrant brain development during sensitive stages without leading to altered neurotransmitter levels in adulthood. Further investigation into the relationship between regional 5-HT changes or 5-HT receptor effects after MDMA exposure may be revealing.

The results of the MK-801 locomotor challenge suggest that the critical period of MDMA exposure for NMDA-related alterations is from PD 1–10 and PD 6–15. This suggests that developmental MDMA exposure causes decreased activity of the NMDA receptor, which has been shown to be important in learning and memory (Morris, 1989; Morris *et al.* 1986). Interestingly, there were no changes observed in the PD 11–20 group, which showed the largest learning and memory deficits. With no changes seen in the PD 11–20 group, it is difficult to link the alterations observed in NMDA response to the learning and memory deficits. However, it should be noted that the PD 11–20 exposure group showed a trend towards a reduced response to MK-801 in MDMA-treated groups even though it did not reach statistical significance. While using locomotor activity as an indicator of alterations in receptor systems provides valuable insight into neurotransmitter function, it does not provide direct evidence on whether NMDA receptors represent an underlying mechanism of MDMA-induced learning deficits. As locomotor activity and learning are mediated by separate brain regions, it is possible that NMDA changes involved in learning and memory would not materialize in the locomotor task at the same age as for NMDA changes caused by the drug in brain regions controlling MWM or CWM learning. The changes in NMDA-mediated locomotor behaviour suggest, however, that NMDA is altered by

developmental MDMA exposure in some regions and these data do not rule out the possibility of NMDA changes in the hippocampus. Further studies should investigate the role of MDMA exposure on NMDA receptor structure and function in hippocampus as well as in the striatum and the role of NMDA receptors during different exposure periods.

Acknowledgements

This work was supported by NIH project grant DA021394 and training grant T32 ES007051. We gratefully acknowledge the assistance of Mary Moran and Holly Johnson for technical assistance.

Statement of Interest

None.

References

- Broening HW, Morford LL, Inman-Wood SL, Fukumura M, *et al.* (2001). 3,4-methylenedioxymethamphetamine (ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. *Journal of Neuroscience* **21**, 3228–3235.
- Bronson ME, Jiang W, Clark CR, DeRuiter J (1994). Effects of designer drugs on the chicken embryo and 1-day-old chicken. *Brain Research Bulletin* **34**, 143–150.
- Cain DP (1997). Prior non-spatial pretraining eliminates sensorimotor disturbances and impairments in water maze learning caused by diazepam. *Psychopharmacology (Berlin)* **130**, 313–319.
- Clancy B, Finlay BL, Darlington RB, Anand KJ (2007a). Extrapolating brain development from experimental species to humans. *Neurotoxicology* **28**, 931–937.
- Clancy B, Kersh B, Hyde J, Darlington RB, *et al.* (2007b). Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* **5**, 79–94.
- Cohen MA, Skelton MR, Schaefer TL, Gudelsky GA, *et al.* (2005). Learning and memory after neonatal exposure to 3,4-methylenedioxymethamphetamine (ecstasy) in rats: Interaction with exposure in adulthood. *Synapse* **57**, 148–159.
- Grace CE, Schaefer TL, Graham DL, Skelton MR, *et al.* (2010). Effects of inhibiting neonatal methamphetamine-induced corticosterone release in rats by adrenal autotransplantation on later learning, memory, and plasma corticosterone levels. *International Journal of Developmental Neuroscience* **28**, 331–342.
- Herring NR, Schaefer TL, Gudelsky GA, Vorhees CV, *et al.* (2008). Effect of (+)-methamphetamine on path integration learning, novel object recognition, and neurotoxicity in rats. *Psychopharmacology (Berlin)* **199**, 637–650.
- Ho E, Karimi-Tabesh L, Koren G (2001). Characteristics of pregnant women who use ecstasy (3, 4-methylenedioxymethamphetamine). *Neurotoxicology and Teratology* **23**, 561–567.
- Kirk RE (1995). *Experimental Design*, 3rd edn. Pacific Grove: California Brooks/Cole Publishing Company.
- Moore DG, Turner JD, Parrott AC, Goodwin JE, *et al.* (2010). During pregnancy, recreational drug-using women stop taking ecstasy (3,4-methylenedioxy-N-methylamphetamine) and reduce alcohol consumption, but continue to smoke tobacco and cannabis: initial findings from the Development and Infancy Study. *Journal of Psychopharmacology* **24**, 1403–1410.
- Morris RG (1989). Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation *in vivo* by the N-methyl-D-aspartate receptor antagonist AP5. *Journal of Neuroscience* **9**, 3040–3057.
- Morris RG, Anderson E, Lynch GS, Baudry M (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* **319**, 774–776.
- Rice D, Barone S (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives* **108** (Suppl. 3), 511–533.
- Saucier D, Hargreaves EL, Boon F, Vanderwolf CH, *et al.* (1996). Detailed behavioral analysis of water maze acquisition under systemic NMDA or muscarinic antagonism: nonspatial pretraining eliminates spatial learning deficits. *Behavioral Neuroscience* **110**, 103–116.
- Skelton MR, Schaefer TL, Herring NR, Grace CE, *et al.* (2009). Comparison of the developmental effects of 5-methoxy-N,N-diisopropyltryptamine (Foxy) to (±)-3,4-methylenedioxymethamphetamine (ecstasy) in rats. *Psychopharmacology (Berlin)* **204**, 287–297.
- Skelton MR, Williams MT, Vorhees CV (2006). Treatment with MDMA from P11–20 disrupts spatial learning and path integration learning in adolescent rats but only spatial learning in older rats. *Psychopharmacology (Berlin)* **189**, 307–318.
- Skelton MR, Williams MT, Vorhees CV (2008). Developmental effects of 3,4-methylenedioxymethamphetamine: a review. *Behavioral Pharmacology* **19**, 91–111.
- Sodhi MS, Sanders-Bush E (2004). Serotonin and brain development. *International Reviews of Neurobiology* **59**, 111–174.
- Vorhees CV (1987). Maze learning in rats: a comparison of performance in two water mazes in progeny prenatally exposed to different doses of phenytoin. *Neurotoxicology and Teratology* **9**, 235–241.
- Vorhees CV, Inman-Wood SL, Morford LL, Broening HW, *et al.* (2000). Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. *Journal of Neuroscience* **20**, 4732–4739.

- Vorhees CV, Reed TM, Skelton MR, Williams MT (2004).** Exposure to 3,4-methylenedioxymethamphetamine (MDMA) on postnatal days 11–20 induces reference but not working memory deficits in the Morris water maze in rats: implications of prior learning. *International Journal of Developmental Neuroscience* **22**, 247–259.
- Vorhees CV, Schaefer TL, Skelton MR, Grace CE, et al. (2009).** (±)3,4-Methylenedioxymethamphetamine (MDMA) dose-dependently impairs spatial learning in the morris water maze after exposure of rats to different five-day intervals from birth to postnatal day twenty. *Developmental Neuroscience* **31**, 107–120.
- Vorhees CV, Schaefer TL, Williams MT (2007).** Developmental effects of ±-methylenedioxy-methamphetamine on spatial vs. path integration learning: effects of dose distribution. *Synapse* **61**, 488–499.
- Vorhees CV, Williams MT (2006).** Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols* **1**, 848–858.
- Williams MT, Morford LL, Wood SL, Rock SL, et al. (2003).** Developmental 3,4-methylenedioxymethamphetamine (MDMA) impairs sequential and spatial but not cued learning independent of growth, litter effects or injection stress. *Brain Research* **968**, 89–101.
- Williams MT, Vorhees CV, Boon F, Saber AJ, et al. (2002).** Methamphetamine exposure from postnatal day 11 to 20 causes impairments in both behavioral strategies and spatial learning in adult rats. *Brain Research* **958**, 312–321.