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Effects of MDMA on olfactory memory and reversal learning in rats

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ABSTRACT

The effects of acute and sub-chronic MDMA were assessed using a procedure designed to test rodent working memory capacity: the odor span task (OST). Rats were trained to select an odor that they had not previously encountered within the current session, and the number of odors to remember was incremented up to 24 during the course of each session. In order to separate drug effects on the OST from more general performance impairment, a simple olfactory discrimination was also assessed in each session. In Experiment 1, acute doses of MDMA were administered prior to select sessions. MDMA impaired memory span in a dose-dependent fashion, but impairment was seen only at doses (1.8 and 3.0 mg/kg) that also increased response omissions on both the simple discrimination and the OST. In Experiment 2, a sub-chronic regimen of MDMA (10.0 mg/kg, twice daily over four days) was administered after OST training. There was no evidence of reduced memory span following sub-chronic MDMA, but a temporary increase in omission errors on the OST was observed. In addition, rats exposed to sub-chronic MDMA showed delayed learning when the simple discrimination was reversed. Overall, the disruptive effects of both acute and sub-chronic MDMA appeared to be due to non-mnemonic processes, rather than effects on specific memory functions.

1. Introduction

Research with recreational ecstasy users has revealed deficits on a number of cognitive tasks. A history of heavy ecstasy use is associated with impaired performance on tests of attention, learning and working memory with simple cognitive tasks (e.g., reaction time) often unaffected, and more complex tasks involving higher processing loads more severely affected (Montgomery & Fisk, 2008; Murphy, Wareing, Fisk, & Montgomery, 2009; Nulsen, Fox, & Hammond, 2010; Parrott, 2013). Of course, these studies have many limitations including the accuracy of the self-reported drug histories on which they are based and the complication that most ecstasy users are also multiple drug users. Further, pills believed by users to be ecstasy may or may not contain only MDMA (Sherlock, Wolff, Hay, & Conner, 1999). Thus, it is difficult to determine whether the differences between controls and ecstasy users are actually based on MDMA use. Indeed, when groups of ecstasy users are compared with groups of participants who do not use ecstasy, but are matched with respect to use of marijuana or other drugs, several studies have found comparable cognitive deficits (e.g., Croft, Mackay, Mills, & Gruzelier, 2001; Dafters, Hoski, & Talbot, 2004; de Sola et al., 2008), although others have found more severe deficits in ecstasy users (Daumann et al., 2005; Nulsen et al., 2010). Due to these difficulties in interpretation and given the ethical restrictions associated with administering MDMA to humans, preclinical studies using non-human subjects, particularly rodents, have an important role in the investigation of these cognitive disruptions.

Numerous studies have shown that acute MDMA administration can impair performance on learning and working memory tasks in rodents (e.g., Arias-Cavieres et al., 2010; Braida, Pozzi, Cavallini, & Sala, 2002; Byrne, Baker, & Poling, 2000; Galizio, McKinney, Cerutti, & Pitts, 2009; Galizio, Byrd, Robinson, Hawkey, & Rayburn-Reeves, 2014; Harper, Wisnewski, Hunt, & Schenk, 2005; Marston, Reid, Lawrence, Olverman, & Butcher, 1999). However, whether these disruptions are specific to working memory processes is not clear. For example, Harper et al. (2005) showed that MDMA effects on delayed matching-to-sample were independent of delay, that is, comparable levels of disruption were observed under conditions of no delay (which presumably do not involve working memory), as well as with delays. Similarly, Galizio et al. (2014) found that MDMA increased latency to locate the hidden platform in the Morris Swim Task, but only at doses that also impaired overall perceptual-motor ability. Finally, Kay, Harper, and Hunt (2010) found that acute doses of MDMA impaired performance on a reference memory version of the radial

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arm maze at doses that had no effect on the working memory version of the task.

There have also been a number of efforts to model the cognitive effects of sub-chronic or binge MDMA use in animals. In these studies, high doses of MDMA are generally administered twice daily for four or more days and the residual effects of the drug regimen on learning and memory are then studied. Within this literature, there are some discrepancies as to the nature of binge MDMA effects. Some studies have found impairments in working (e.g., Marston et al., 1999) or recognition memory (e.g. Camarasa, Marimon, Rodrigo, Escubedo, & Pubill, 2008) tasks, while others have found impairments in reference memory task acquisition (e.g. Skelton et al., 2008), retention (e.g. Able, Gudelsky, Vorhees, & Williams, 2006), or both (Cunningham, Raudensky, Tonkiss, & Yamamoto, 2009). Based on these and similar findings, some have questioned whether impaired performance on classic working memory tasks reflects specific working memory deficits or more general cognitive impairments (Kay, Harper, & Hunt, 2011). Additionally, results have been mixed with several studies failing to observe any cognitive deficits following binge MDMA exposure (e.g., Byrne et al., 2000; Slikker et al., 1989).

Working memory tasks in rodents are typically characterized by remembering a stimulus or place within a single trial or session, but not over longer durations or between sessions (Dudchenko, 2004). However, human models of working memory also emphasize its limited capacity (Baddeley, 1986; Cowan, Chen, & Rouder, 2004), meaning that the number of items to be remembered is a key determinant of memory accuracy, and as noted, the deficits observed in human MDMA users seemed linked to those tasks which involve high memory demands (Parrott, 2013). Relevant to this point, the rodent odor span task (OST) can be used to study memory of varying numbers of stimuli (Dudchenko, Wood, & Eichenbaum, 2000). The OST is an adaptation of the delayed non-match to sample task in which the rats are presented with a series of odors and only responses to new odors are rewarded. Unlike the standard delayed non-match-to-sample task, once a sample has been presented, it serves as a sample for all subsequent trials. This allows the number of samples to accumulate over the course of the session, meaning that accurate responding is based on a steadily increasing number of remembered items. This feature is unique to the OST and for this reason it was nominated as the task to model memory capacity by the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) group (Dudchenko, Talpos, Young, & Baxter, 2013).

At this point though, there are only a few studies on the behavioral pharmacology of the OST. Several studies have found that NMDA antagonists such as MK-801 and ketamine can impair accuracy in the OST and that nicotine can enhance it (Galizio, Deal, Hawkey, & April, 2013; MacQueen, Bullard, & Galizio, 2011; Rushforth, Steckler, & Shoaib, 2011), but no research with stimulant drugs other than nicotine has been published using this procedure. The present study used the OST to test the effects of acute and binge MDMA under conditions in which the number of stimuli to remember varied. In Experiment 1, performances were assessed under a range of acute doses of MDMA. In Experiment 2, rats were exposed to binge doses of MDMA or saline. Then any residual impairment on task performance was assessed. In both experiments, an olfactory simple discrimination task was included to measure generalized forms of performance impairment which are unrelated to working memory, but might reduce accuracy on the OST. In Experiment 2, after the initial assessment, a contingency reversal of the simple discrimination was performed to test for effects of binge MDMA on behavioral flexibility. This was done in an attempt to replicate the findings of Kay et al. (2011) who found residual deficits in reversal learning in the radial arm maze following binge exposure to MDMA.

2. Experiment 1: effects of acute MDMA on OST performance

2.1. Method

2.1.1. Subjects

Subjects were six male Sprague-Dawley (Harlan) rats. All subjects were between 90 and 150 days old at the beginning of testing. Rats were individually housed in a temperature and humidity controlled vivarium on a 12/12 h light–dark cycle. Water was continuously accessible in the home cage and food access was restricted to maintain each rat at approximately 85% of its free-feeding weight.

2.1.2. Apparatus/stimuli

Olfactory span training and testing took place in a circular open-field apparatus. This apparatus consisted of a circular table 94 cm in diameter bordered by a wall of sheet metal baffling. The surface of the table contained eighteen holes positioned in two concentric circles. Plastic cups (2 oz) were placed in each hole during session trials. Speakers adjacent to the span arena provided white noise (70 dB) during all sessions. A web cam (Logitech, Inc.) was used to digitally record each session.

All stimuli consisted of plastic cups half filled with fine grained, white, play sand and covered by scented plastic lids. The lids were scented by storing them in airtight plastic containers containing household spices and flavorings (e.g. oregano, nutmeg, etc.—see Galizio et al., 2013 for a complete list of odorants). These scented lids were placed lightly on the stimulus cups for each trial and were exchanged for unused lids prior to each presentation of a given scent.

2.1.3. Initial training

Subjects were tested five sessions per week (daily, Monday–Friday) throughout training and testing. At first exposure to the arena, cups containing sugar pellets (45 mg Bio-Serv) were presented. Once pellets were readily consumed from these cups, trials were conducted where the baited cups were presented with an unscented plastic lid partially covering the opening. The position of the lid was gradually shifted to cover the opening of the cup completely. Once the rat was reliably removing the unscented lid to retrieve the sucrose pellet, odor training began.

2.1.4. Odor span training

The current study used an OST procedure adapted for behavioral pharmacology (Galizio et al., 2013) illustrated in the top row of Fig. 1. On trial 1, a single olfactory stimulus (A) was presented and marked the location of a reinforcer (+). Removal of this lid allowed access to the pellet inside the cup. On trial 2, the previous odor (A−) and a novel odor (B) were presented and the novel odor marked the location of the reinforcer (B+). On trial 3, the two previously presented stimuli were presented (A−, B−), as was a new odor (C). Again, the novel stimulus indicated the location of the reinforcer (C+). This pattern continued for subsequent trials, but for all trials after the fifth, the novel scent was presented with four comparison scents pseudo-randomly selected from the pool of previously presented scents (see Trial N of Fig. 1). This was designed to eliminate the confound between the number of comparison stimuli in the arena and the number of stimuli to remember which is present in some previous OST studies (e.g., Dudchenko et al., 2000).

OST trials were presented on a multiple schedule in each testing session with trials of an olfactory simple discrimination (SD) task (see Fig. 1, bottom row). The SD procedure used five odors which were not included in the pool of odors for span trials, one of which was designated as S+(ex. bubblegum) and four were designated as

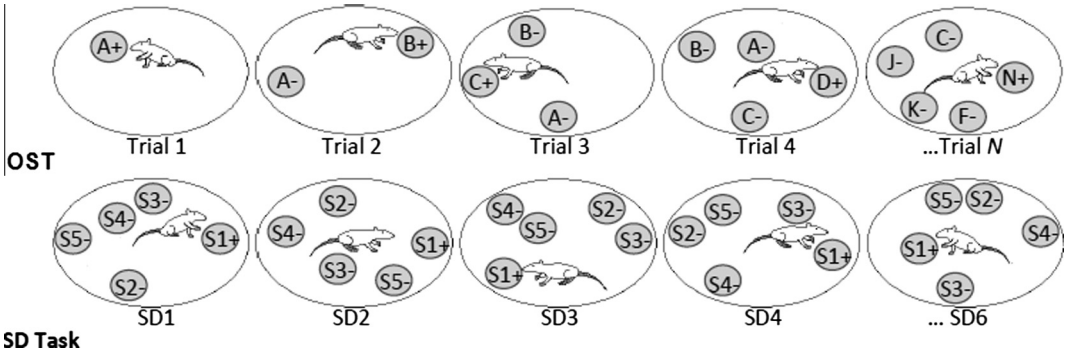

Fig. 1. Cartoon depicting procedures for the OST (top) and SD (bottom) tasks.

S−(ex. grape, cherry, vanilla, almond). Responses to S+ were always reinforced and responses to S− were never reinforced. The logic of this control is that SD trials require the same level of motor function, olfactory perception, and motivation to complete as OST trials, but do not require remembering whether stimuli have been presented within the current session (working memory), and so can assess potential non-mnemonic effects of a drug such as MDMA.

Typical sessions consisted of 30 experimental trials (24 OST trials and 6 SD control trials, described below) separated by an inter-trial interval of approximately one minute. Each trial began when the subject was placed in the arena and was completed when a correct response was made or after two minutes without a response. A response was defined as the removal of a lid from a stimulus cup using the front paws or snout. A correct response occurred when the first lid removed was the novel odor (S+), and a correction procedure allowed the session to continue after an incorrect response (removing the lid of a previously presented odor) until the S+ lid was removed and a sugar pellet reward was retrieved. If two minutes elapsed without a response, an omission was recorded and the rat was given an additional trial where only the S+ of an omitted trial was present in the arena. If the two minute trial termination criterion was met six times within the session, the session was terminated and all remaining trials were scored as omissions. Location of the S+ and S− stimulus was determined randomly on each trial.

2.1.5. Measures

Across a session, percent correct, span, longest run and omissions were the primary measures. The accuracy of the first response in each trial was noted and compiled into an overall measure of percent correct (e.g. 18 correct responses/24 total OST trials = 75% Correct). Span refers to the number of trials completed before the first error of any kind (Trial 1 was not included as there are no stimuli to remember at this point). An additional measure of consecutive correct responses was longest run, identified as the longest series of correct responses within a session. Omission errors occurred when two minutes elapsed within a trial without a response. Omission errors were excluded from percent correct calculations and were used to generate a percent omission score, reflecting the percentage of trials on which no response occurred.

2.1.6. Additional controls

In addition to the SD trials, several important controls were included. To ensure that subjects could not smell their previous contact with the lids, each scented plastic lid was only used for a single presentation of that odor within each session. In addition, trials were periodically presented without sucrose pellets present to assess whether the odor of the pellet was detectable. Accuracy on these trials did not differ from baited trials, as determined by a 95% confidence interval around the baseline mean ($p > .05$). To assess any bias in experimenter scoring, a sample of testing session videos were selected and scored by a second experimenter blind to the condition and baited cup. Inter-rater agreement was high (99%).

2.1.7. Drug phase

Prior to the drug phase, all subjects were required to meet a stability criterion on percent correct for both the OST and SD tasks. Within the previous sessions, the difference in mean percent correct for the most recent five sessions and previous five sessions could not exceed 10% of the grand mean of the ten sessions (Mean of sessions 1–5 − Mean of sessions 6–10 < .10 × Mean of sessions 1–10). Once this criterion was met, drug testing began.

Subjects were administered (I.P.) one of a range of acute doses of MDMA (0.3, 1.0, 1.8, 3.0 mg/kg), saline, or baseline (no injection) prior to daily testing. These doses were selected in order to characterize the full range of MDMA effects from a dose that was low enough to be without behavioral action through a dose high enough to produce general performance deficits based on previous research (e.g., Kay et al., 2010). Each week, two days served as baseline testing days, where no injections were received. One day each week, subjects received an injection of saline 15 min prior to testing. On the two remaining days, an acute dose of MDMA was administered 15 min prior to testing. MDMA hydrochloride (NIDA) was dissolved in physiological saline and injected in a volume of 1.0 ml/kg. Doses were determined two to four times for each rat and were administered in a semi-random order. In most (5/6) cases, subjects were drug-naive prior to beginning the acute MDMA study; one animal participated in a similar acute drug study with ketamine prior to MDMA testing. In this case, the subject completed two weeks of testing without injections between drug phases to ensure there were no carry-over effects.

2.2. Data analysis

All data analysis was performed using SPSS version 20. Span and longest run were analyzed using separate 1 (measure) × 6 (condition) ANOVA tests. Task (OST vs. SD) × condition (baseline, saline, 0.3, 1.0, 1.8, 3.0 mg/kg MDMA) effects were assessed using 2 × 6 ANOVA. Due to major violations of assumptions for parametric tests, omissions were analyzed using non-parametric analysis. Unplanned *post hoc* tests were performed using Tukey correction for multiple tests, unless specified otherwise.

2.3. Results

Fig. 2 shows the effects of MDMA on the key dependent variables in this study: span, longest run, percent correct, and omissions. The top panel plots two measures of consecutive correct

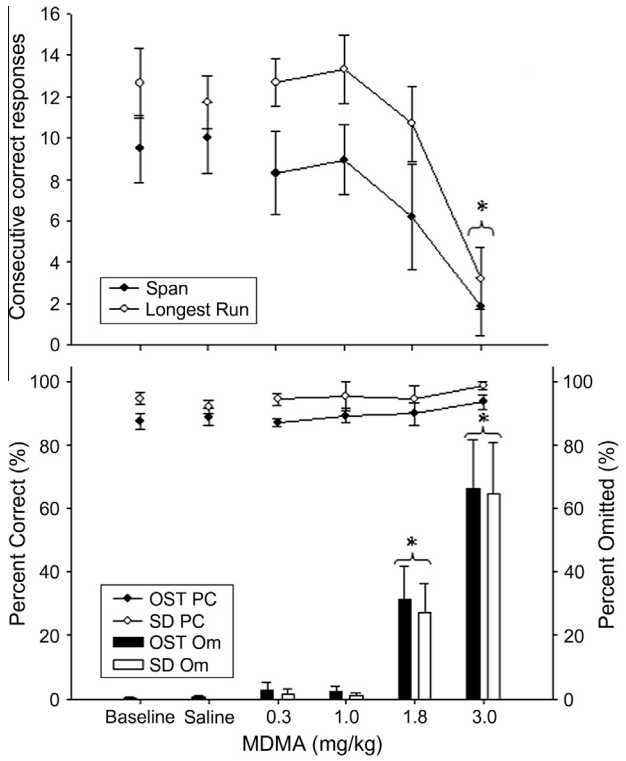

Fig. 2. Dose-response effects of acute MDMA on primary measures. Top panel shows mean span and longest run as a function of MDMA dose. The bottom panel shows mean percent correct (PC) and percent of response omission trials (Om) on the OST and SD tasks. For PC, 3.0 mg/kg was excluded from analyses due to reduced sample size due to omissions. Bars indicate percent omissions by task. All error bars indicate SEM. Asterisks (*) indicate significant differences from saline.

responses: span (black circles) and longest run (white circles). Under baseline and control conditions, mean spans averaged 9–10 odors, whereas longest runs were somewhat higher with runs of 11–12. MDMA produced dose dependent reductions in both span [$F(5,25) = 7.33$, $p < .05$] and longest run [$F(5,25) = 10.59$, $p < .05$]. *Post hoc* tests confirmed that both span and longest run were significantly below saline levels at the 3.0 mg/kg dose of ($p < .05$), but not at lower doses ($p > .05$). This pattern indicates that a high dose of MDMA reduced the number of consecutive correct responses in the OST, but further analyses are required to interpret this effect.

The lower panel shows percent correct for the full session as a function of MDMA dose for the OST (black circles) and SD (white circles) tasks. Under baseline, saline, and all MDMA conditions, accuracies in both tasks were quite high, approaching 100% on SD trials and only slightly lower, nearly 90%, on the OST. Percent correct was calculated according to all trials in which a response occurred, so errors of omission were excluded. A significant between-task difference was detected [$F(1,5) = 9.36$, $p < .05$] which indicated that percent correct was higher on the SD than the OST. However, percent correct was not significantly affected by MDMA dose [$F(4,20) = 0.16$, $p > .05$], and no significant interaction between task and dose was found [$F(4,20) = 0.38$, $p > .05$]. Although response accuracy was insensitive to impairments produced by acute MDMA, higher doses of the drug did affect performance. Bars in the lower panel indicate the percentage of trials which were omitted due to non-responding on the OST (black bars) and the SD (white bars). Response omissions were quite infrequent during baseline, saline and low dose conditions, but became more frequent as the MDMA dose increased. At the highest MDMA dose (3.0 mg/kg), omissions accounted for the majority of trials. Non-parametric Freidman ANOVAs yielded significant effects in the OST, ($X^2(4) = 120.7$, $p < .01$) and the SD task ($X^2(4) = 112.1$, $p < .01$). Increases in omissions occurred at the 1.8 and 3.0 mg/kg doses in both tasks. This outcome provides an account of the consecutive correct response data: spans and longest runs were lower after 3.0 mg/kg MDMA not because of increases in selecting incorrect odors, but rather because of the increase in response omissions. In summary, acute MDMA produced a dose-dependent disruption in performance by increasing errors of omission. This effect was similar in both tasks, and these omissions resulted in the reduced spans and longest runs, indicating that this impairment was unrelated to specific memory requirements.

Fig. 3 shows a comparison of accuracy as a function of number of stimuli to remember (memory load) in the saline (black circles) and 1.8 mg/kg MDMA (white circles) conditions on the OST. The 1.8 mg/kg dose was selected because it was the lowest dose of MDMA which produced a significant impairment on performance. In the Dose × Memory load analysis, no significant effects were observed for dose [$F(1,5) = .027$, $p < .05$], a significant effect was found for memory load [$F(5,25) = 3.440$, $p < .05$], and no interaction was found [$F(5,25) = .425$, $p > .05$]. Accuracy on the OST declined as the number of stimuli increased, but MDMA did not alter this function.

In summary, acute MDMA produced dose-dependent impairments of performance on the OST and the olfactory SD task. However, these disruptions in the OST were evident only at doses that produced large numbers of response omissions on both tasks and thus, are best interpreted as performance disruptions, rather than effects on remembering.

3. Experiment 2: effects of sub-chronic MDMA on OST performance

Although acute exposure to MDMA did not produce amnestic effects in Experiment 1, concerns about MDMA toxicity have focused on residual effects on memory after binge or sub-chronic use of MDMA. Experiment 2 sought to assess the impact of sub-chronic MDMA exposure on performance in the OST and a SD task, as well as in a performance reversal.

3.1. Method

3.1.1. Subjects and apparatus

Subjects were 12 experimentally-naïve male Sprague-Dawley rats between 90 and 150 days old at the onset of the experiment.

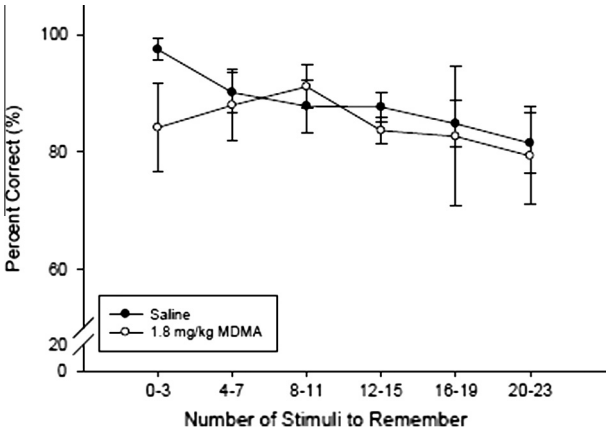

Fig. 3. Within-session accuracy. Percent correct as a function of memory load (number of stimuli to remember) compared between saline and 1.8 mg/kg MDMA conditions. Error bars indicate SEM.

Housing conditions and experimental apparatus were the same as in Experiment 1.

3.1.2. Procedure

All pre-drug training and testing proceeded as described in Experiment 1. Once subjects met stability criteria, they began the sub-chronic drug phase on the following Monday. Injections (I.P.) of either MDMA (10.0 mg/kg, twice daily over four days) or saline were administered from Monday to Thursday. This administration schedule has been shown in previous experiments to produce persistent alterations in serotonergic and behavioral function (e.g. Battaglia, Yeh, & De Souza, 1988; Reneman et al., 2002; Robinson, Castaneda, & Whishaw, 1993). A 72-h recovery period followed the final injection of the series, and rats were then tested on the OST and SD tasks for 10 sessions.

After the completion of these 10 sessions, an additional 10 sessions were conducted with the SD contingency reversed, i.e., the previous S+(ex. bubblegum) no longer contained the reinforcer. Instead, one of the four negative stimuli was randomly selected (e.g., cherry) to serve as the new S+. For two subjects in each condition, the reversed SD trials were interspersed among the OST trials as in previous training. All additional subjects ($n = 4$ per condition) completed the six reversed SD trials following the end of OST testing. This was done to enhance acquisition of the SD within the limited testing window of 10 sessions.

3.1.3. Data analysis

Analyses were conducted as in Experiment 1 except that between × within subject ANOVAs were used to address the effect of condition and phase of testing. In some cases the assumption of sphericity was violated, so scores and degrees of freedom were adjusted using the Greenhouse–Geisser correction (Greenhouse & Geisser, 1959). In most cases, this resulted in reported degrees of freedom which are not whole integers. As in Experiment 1, omission data violated key assumptions for parametric tests, so omissions were analyzed using non-parametric analysis. Reversal acquisition curves for MDMA- and saline-treated animals were compared using trend analysis (GLM linear contrasts).

3.2. Results

Table 1 and Fig. 4 show the effect of chronic treatments of MDMA or saline on the key dependent measures in the current study: span, longest run, percent correct responses and percent response omissions. Table 1 shows mean span and longest run for MDMA- and saline-treated animals over three phases of testing: baseline, post-binge, and post-reversal. Separate 2 × 3 ANOVA tests (2 conditions × 3 phases) were performed for span and longest run. For span, no significant effect was detected for drug condition [$F(1,10) = 1.764$, $p > .05$] or for testing phase [$F(2,20) = .043$, $p > .05$]. There was also no interaction [$F(1,10) = 1.093$, $p > .05$]. Similarly, for longest run, no significant effect was detected for drug condition [$F(1,5) = .027$, $p > .05$] or for testing phase [$F(1.279,12.791) = .389$, $p > .05$] and there was no interaction [$F(1.279,12.791) = .846$, $p > .05$]. While longest runs tended to be longer than span, these two measures of consecutive correct responses were stable throughout testing and were found to be insensitive to the effects of chronic treatments of MDMA.

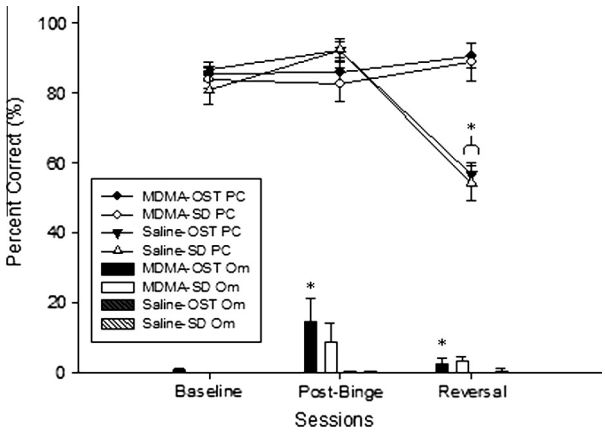

Fig. 4. Percent correct responses and omissions across testing phases. Percent correct responses (PC, lines) and response omissions (Om, bars) over testing phases. All error bars indicate SEM. Asterisks (*) indicate significant differences from baseline.

Fig. 4 shows percent correct and omissions for Experiment 2. The line graph indicates percent correct for saline (triangles) and MDMA-treated (circles) subjects on the OST (black) and the SD task (white). Separate 2 × 3 ANOVA (2 conditions × 3 phases) tests were run on the two tasks. In the OST, there was no significant effect of drug condition [$F(1,10) = .85$, $p > .05$] or testing phase [$F(2,20) = .06$, $p > .05$] and there was no interaction [$F(2,20) = .67$, $p > .05$]. In the SD task, there was a significant effect for testing phase [$F(2,20) = 157.74$, $p < .05$], but not condition [$F(1,10) = .09$, $p > .05$] and there was no interaction [$F(2,20) = .16$, $p > .05$]. Follow-up tests (LSD) indicated that SD percent correct was significantly reduced in the reversal phase relative to baseline ($p < .05$). No other significant differences in percent correct were detected. MDMA treatment did not generally affect the accuracy of responses in either olfactory memory task; the only significant effect noted in accuracy was the expected reduction in simple discrimination (SD) performance following a contingency reversal.

However, sub-chronic MDMA did affect task performance. Bars in Fig. 4 indicate the percent of trials without a valid response for the saline (striped) and MDMA-treated (solid) animals on the OST (dark) and SD (light) tasks. Overall, errors of omission were relatively infrequent across the study; however, omissions appeared more frequently immediately following the binge MDMA treatments and this increase appeared to dissipate by the reversal phase. Separate nonparametric Mann–Whitney U tests were run on the OST and SD tasks. In the OST, significant between-condition effects were found, indicating increases in errors of omission in the post-binge phase ($U = 35.5$, $p < .05$) and reversal ($U = 33$, $p < .05$) in the MDMA-treated group. In the SD task, mean omissions appear elevated following binge MDMA, but these levels failed to reach significance in both the post binge ($U = 25$, $p > .05$) and reversal

Table 1
Consecutive correct responses analyzed by training phase.

Condition	Measure	BL	Error	PB	Error	Rev	Error
MDMA	Span	6.80	±0.97	6.40	±1.03	7.38	±2.00
	LR	11.22	±0.96	9.87	±1.20	11.32	±2.25
Saline	Span	9.08	±1.44	9.68	±1.52	9.10	±2.00
	LR	11.75	±1.08	12.85	±0.95	12.95	±1.47

Table reports mean measures of consecutive correct responses (span and longest run) and SEM for baseline (BL), post-binge (PB), and post-reversal (Rev) phases of testing.

phases ($U = 29$, $p > .05$). In sum, MDMA-treated animals had higher rates of omissions than untreated animals and this increase appeared to be somewhat selective to the OST.

Fig. 5 shows acquisition curves for saline (white circles) and MDMA-treated (black circles) subjects following the discrimination reversal. Both groups showed a substantial reduction in percent correct immediately following the contingency reversal followed by a gradual increase in percent correct over subsequent sessions. Between-group differences in curvilinear acquisition functions were assessed using trend analysis. A significant quadratic interaction was detected [$F(1,10) = 7.740$, $p > .05$], indicating that the curvilinear relationship between response accuracy and testing day is dependent upon drug condition. The greatest difference in the curves occurs during the first two post-reversal sessions. On average, saline-treated animals responded correctly on slightly more than one of the six reversal trials (mean = 17.78%), while MDMA treated animals rarely made a correct response in the first session (mean = 2.78%). By the second session, saline-treated animals improved by an average of one correct response per session (mean = 36.11%), while MDMA-treated animals continued to make less than one correct response in the six presentations (mean = 11.66%). By the third or fourth post-reversal session, accuracies were very similar between the two groups and remained so for the rest of acquisition phase.

4. General discussion

The experiments reported here aimed to test the potential for MDMA-induced impairments in performance on the OST, which is coming to be regarded as the benchmark animal model for memory capacity (Dudchenko et al., 2013). The results showed that both acute and sub-chronic MDMA produced impairments of OST performance, yet, the pattern of task disruption suggests that non-mnemonic processes, rather than specific memory functions, were the basis for the observed effects.

In Experiment 1, a dose dependent reduction in span and longest run was observed. This is a key analysis, as span is a classic measure of working memory function (Dudchenko et al., 2000). In some analyses, this effect would be interpreted as working memory impairment, indicating a reduction in the capacity of working memory. However, this conclusion is inconsistent with the pattern of errors beyond the span measure as reductions in span and longest run occurred only at doses which produced a significant increase in errors of omission, where valid responses failed to occur. Further, the increase in errors of omission was not specific to the OST as simple discrimination performance was impaired equally. Additionally, while task completion was disturbed by acute MDMA on both tasks, the accuracy of responses which did occur was unaffected, indicating that the relevant memory functions were resistant to the disruptions produced by acute MDMA. Measures of consecutive correct responses such as span and longest run are sensitive to disruptions in responding, but traditionally cannot discriminate between different types of errors. In the current study, these classical measures of working memory performance appear to have detected a general behavioral impairment which is unrelated to memory. More specifically, acute MDMA impairs performance by reducing the number of valid responses without reducing the accuracy of responses when they do occur.

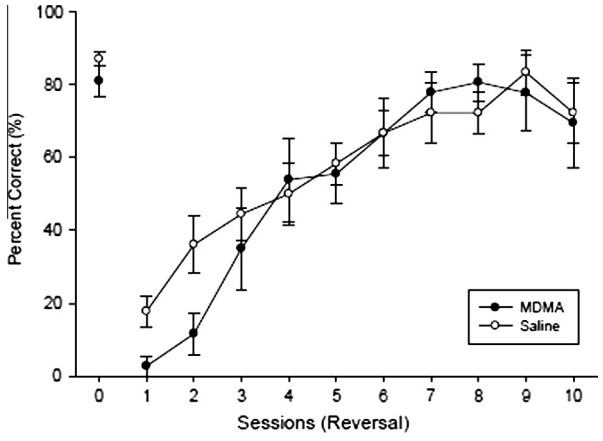

Fig. 5. Reversal acquisition. Acquisition curves for saline- and MDMA-treated subjects following SD reversal. Baseline values are mean SD accuracies for the last 10 sessions prior to treatment. Error bars indicate SEM.

The pattern of impairment observed in Experiment 1 is striking, but was unexpected given the existing literature on acute MDMA in rats. Previous studies utilizing measures of accuracy often observed acute reductions of accuracy in working memory tasks (e.g. Braida et al., 2002; Galizio et al., 2009; Young, McGregor, & Mallet, 2005), reference memory tasks (e.g. Kay et al., 2010), or both. In some cases, the effects of acute MDMA were attributed to more general or non-mnemonic causes (e.g. Harper et al., 2005; Marston et al., 1999), but even in these cases, increases in committed errors were observed. The differences between the current paradigm and these previous tasks may offer an explanation for the current failure of MDMA to produce committed errors.

One possible explanation is that the tendency of MDMA to produce omissions or committed errors depends upon the apparatus used. Operant chamber tasks, such as non-match to position (e.g. Galizio et al., 2009; Harper et al., 2005), and mazes with confined pathways, such as the radial arm maze (e.g. Braida et al., 2002; Kay et al., 2010) tend to find increases in committed errors. However, in larger, open designs, such as the Morris Swim Task (Galizio et al., 2014), it has been reported that acute MDMA impairs performance by interfering with the learned response itself. Galizio et al. (2014) reported that high doses of MDMA altered the path taken to approach a hidden platform in the water maze and frequently resulted in response failures. The authors suggested that this effect could be due to behavioral alterations which are incompatible with efficient navigation of the maze, such as thigmotaxis or motor deficits. Such stereotypic behavioral alterations following MDMA administration have been well documented previously in confined or open field tasks (e.g. Byrne et al., 2000; Marston et al., 1999; Spanos & Yamamoto, 1989). Stereotypic behavior which is incompatible with completion of the task could have been responsible for the increase in errors of omission while preserving the accuracy of responses which did occur in the OST as well.

Alternatively, the pattern of task disruption could be unique in the current study due to more fundamental differences between the discriminations required to complete the task. All known past studies of acute MDMA have required rats to remember and discriminate between places or positions. Instead, the current study utilized olfactory memory tasks which rely on the rodent's most developed discriminative modality and, as a result, the high salience and discriminability of olfactory stimuli may have decreased the likelihood of a committed error under acute MDMA. Indeed, accuracy of responding remained quite high across all doses of MDMA in both the OST and SD conditions. This account is consistent with previous findings from Harper and colleagues (Harper et al., 2005; Harper, Hunt, & Schenk, 2006) that MDMA-induced errors can be attenuated by increasing the discriminability of relevant cues and more general findings that behavior under strong stimulus control is less sensitive to drug effects (Katz, 1982; Katz, 1983). Such an account is also consistent with more general notions about the role of task difficulty in its sensitivity to MDMA in human populations (e.g. Nulsen et al., 2010).

In Experiment 2, binge MDMA failed to produce measurable differences in span, longest run and response accuracy on the OST and SD tasks relative to non-treated saline controls. Rather, this administration schedule appeared to produce an elevation in rates of omission errors in the OST, though this effect was not sufficient to significantly alter span or longest run. Taken together, it can be concluded that binge MDMA does produce a small, but significant deficit in OST/SD performance, but that this effect impacted task completion rather than accuracy. Although binge MDMA failed to alter response accuracy on tasks where subjects received extensive pre-training, it did alter percent correct in a discrimination reversal. MDMA-treated subjects made fewer correct responses than controls during the first two sessions following the reversal, but acquired the reversed contingency at a similar rate and mastery as training continued. This indicates that initially, these rats perseverated in the previously trained response pattern to a greater degree than controls. This finding replicated the results of Kay et al. (2011) who found that binge MDMA impaired reversal learning in a version of the radial arm maze. This suggests that impairment of reversal learning following binge MDMA is not modality-specific, as it can be demonstrated in both spatial (Kay et al.) and non-spatial (the present study) tasks.

The finding of delays in reversal learning following sub-chronic MDMA is also consistent with numerous studies showing cognitive deficits in humans with histories of heavy MDMA use (c.f., Parrott, 2013). However, studies with these same populations have frequently reported deficits on working memory among MDMA users, as well (Parrott, 2013). In contrast, the present study found that neither acute nor sub-chronic MDMA produced specific effects on working memory capacity in the OST. The absence of support for a working memory effect of MDMA may be viewed as consistent with the argument that factors other than MDMA use *per se* may be responsible for memory deficits in humans. Of course, it should be acknowledged that most of the human research has studied memory for visual stimuli and olfactory memory processes involve different neural pathways and possess some unique features. For example, Dudchenko et al. (2000) found that hippocampal lesions disrupted performance on a spatial span task, but not on the OST in rats.

Alternatively, the translational interpretation of the OST could be questioned. In terms of face validity, the OST is arguably the animal task that most resembles tasks used to assess memory capacity in humans (Dudchenko et al., 2013). It should also be noted that the OST has been shown to be sensitive to amnestic drugs other than MDMA. For example, several studies have shown that NMDA antagonists can produce selective impairments in span and OST accuracy (Galizio et al., 2013; MacQueen et al., 2011). Interestingly however, recent research from our laboratory has shown that rats can perform at above chance levels with up to 72 different odors on this task (April, Bruce, & Galizio, 2013). These data suggest a memory capacity that is more consistent with processes other than working memory, at least as conventionally studied in humans. At present, the understanding of neurobiological variables affecting OST is quite limited and more research is needed to clarify the implications of findings from this animal model for human memory.

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