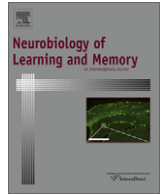




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The effects of binge MDMA on acquisition and reversal learning in a radial-arm maze task

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ABSTRACT

The current study used the partially-baited radial-arm maze paradigm to study the effects of a single-treatment high-dose exposure ('binge') to MDMA (\pm 3,4-methylenedioxymethamphetamine or 'Ecstasy') on memory task acquisition. Sprague–Dawley rats were administered a binge dose (4×10 mg/kg) of MDMA and their ability to subsequently acquire the radial-arm maze task was compared against saline controls. The MDMA-treated rats were significantly slower to learn the task and made more reference memory errors than the controls. Working memory function was found to be relatively unimpaired. Following a reversal of task rules the MDMA-treated rats were again significantly slower to acquire the appropriate rule despite having eventually achieved a similar level of overall performance as control rats. However evidence of drug tolerance was found when all rats were challenged with an acute low dose of MDMA (1×4.0 mg/kg) because the binge MDMA rats were relatively less impaired. Therefore, although binge treated MDMA rats were able to achieve very accurate performance equivalent to the controls they took significantly longer to do this and were less able to adapt their behavior to a change in task rules. In addition the binge treated MDMA rats displayed tolerance to acute MDMA exposure. These findings are consistent with the possibility that human Ecstasy users may show deficits in acquiring information and may experience deficits in cognitive flexibility as well as developing tolerance to the drug with repeated exposure.

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1. Introduction

Although recreational MDMA (\pm 3,4-methylenedioxymethamphetamine or 'Ecstasy') use causes a range of positive behavioral and emotional changes, users also demonstrate general mental confusion (Davison & Parrott, 1997) and significant impairments on a variety of cognitive tasks (Heffernan, Ling & Scholey, 2001). As well as general memory deficits (Rodgers, 2000) there are more specific areas of cognitive function that are impaired with Ecstasy use such as the ability to learn and recall verbal information (e.g., Bolla, McCann, & Ricaurte, 1998; McCardle, Luebbers, Carter, Croft, & Stough, 2004; Morgan, McFie, Fleetwood, & Robinson, 2002; Parrott & Lasky, 1998; Rodgers, 2000; Smith, Tivarus, Campbell, Hillier, & Beversdorf, 2006). Ecstasy use has also been associated with impairments in various tasks that assess executive functioning (e.g., Wareing, Fisk, & Murphy, 2000; Fox, Parrott & Turner, 2001; Heffernan, Jarvis, Rodgers, Scholey & Ling, 2001; Montgomery, Fisk, & Newcombe, 2005; Zakzanis & Young, 2001) including tasks that are used to assess working memory (Fox et al., 2001;

Morgan et al., 2002; VonGeusau, Stalenhoef, Huizinga, Snel, & Ridderinkhof, 2004; Wareing, Murphy, & Fisk, 2004).

However as noted by a number of researchers the findings from Ecstasy users are confounded by a number of variables such as the purity of Ecstasy tablets, the amount used, pre-existing cognitive impairments, the self report measures used and polydrug use. These factors make determining whether MDMA actually causes cognitive impairments difficult and they also make establishing the exact nature of the cognitive impairments seen in Ecstasy users difficult to ascertain. Animal studies help resolve these issues as they provide a much greater degree of experimental control.

Many different kinds of tasks have been used to assess the effects of chronic and binge MDMA administration on cognition in animal models. Although acute MDMA exposure has been found to impair performance on delayed matching to sample (DMTS) tasks in a variety of species (Frederick, Gillam, Allen, & Paule, 1995; Harper, Hunt, & Schenk, 2006; Harper, Wisniewski, Hunt, & Schenk, 2005; LeSage, Clark, & Poling, 1993; Taffe et al., 2001), studies that have examined ongoing performance changes as a result of binge or chronic MDMA exposure have sometimes failed to produce evidence of impairments on these tasks (Frederick et al., 1995, 1998; LeSage et al., 1993; Taffe et al., 2001). However, the absence of an ongoing impairment is not universal. For example, Marston, Reid, Lawrence, Olverman, and Butcher (1999) found a

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binge regime of MDMA impaired DNMT performance during drug administration days and this deficit did not improve 16 days post drug treatment suggesting it had a harmful long-term effect on memory. Therefore the evidence that chronic or binge MDMA exposure produces memory deficits in DMTS-type tasks is mixed, with the majority of studies failing to produce evidence of impairment.

Various maze tasks have been used to assess the effects of chronic and binge doses of MDMA on cognition. Some early studies found that despite binge MDMA treatments producing significant reductions in brain 5-HT they failed to produce evidence of cognitive impairments as assessed using a complex 24 arm maze task (Slikker et al., 1989) or a simpler T-maze task (Ricaurte et al., 1993). However more consistent evidence that MDMA exposure may affect spatial memory comes from other maze tasks such as the Cincinnati water maze (multiple T-maze). Both binge (Able et al., 2003; Skelton et al., 2008) and chronic (Broening, Morford, Inman-Wood, Fukumura, & Vorhees, 2001; Skelton, Williams, & Vorhees, 2006; Skelton et al., 2009; Williams et al., 2003) regimes of MDMA have been shown to impair performance on this maze task indicating MDMA exposure appears to impair spatial memory and path integration processes (Skelton et al., 2008).

In addition, research utilizing the Morris water maze, which is one of the most commonly used mazes to assess spatial memory (D'Hooge and De Deyn, 2001), has found evidence that MDMA exposure can produce deficits in cognitive performance. Researchers have examined the developmental impact of MDMA by administering chronic regimes of MDMA to adolescent rats and examining their ability to acquire water maze tasks in adulthood. These studies have typically found that chronic MDMA exposure impairs performance on water maze tasks (Broening et al., 2001; Skelton et al., 2006, 2009; Vorhees, Reed, Skelton, & Williams, 2004; Williams et al., 2003). Binge MDMA treatments administered in adult rats have also been shown to produce impairments in Morris water maze performance (Robinson, Castaneda, & Whishaw, 1993; Sprague, Preston, Leifheit, & Woodside, 2003; Able et al., 2006; Skelton et al., 2008). Interestingly when examining the different types of Morris water maze tasks it has been found that both binge and chronic MDMA exposure impairs performance on the standard water maze task that it used to assess reference memory processes (Lindner, Balch, & Vandermaelen, 1992) but leaves other forms of the task that assess working memory (Vorhees et al., 2004) and cued memory intact (Broening et al., 2001; Vorhees et al., 2004; Williams et al., 2003). Thus there is evidence that both chronic and short-term binge regimes of MDMA impair reference memory processes more than other memory processes. However it should be noted that the Morris water maze has a potential confound in that it uses the aversive stimulus of being placed in water to motivate the escape behavior of rats. This may be problematic because chronic MDMA exposure had been shown to reduce anxiety in rats (Mechan et al., 2002).

The partially baited radial arm maze paradigm is a particularly useful paradigm as it enables both reference and working memory processes to be examined simultaneously (Olton & Papas, 1979). Using this paradigm the previous research examining the binge and chronic effects of MDMA on Morris water maze performance can be extended by allowing working and reference memory processes to be investigated using the same procedure. To date the only study that has used this apparatus in chronic MDMA research was conducted on rats that were prenatally treated with MDMA and this produced no effect on maze performance in the offspring of these rats when tested in adulthood (Thompson et al., 2009). However, this finding does not answer whether MDMA exposure would affect radial maze performance in rats who are directly administered the drug rather than being exposed via their pregnant mothers.

There is also some question as to the longevity of the impairments seen in MDMA-induced cognitive deficits. While there have been instances where the deficits seen after binge MDMA exposure have been transient (Robinson et al., 1993) there is also some evidence that chronic and binge MDMA exposure have produced more long-term cognitive deficits which remain several weeks after drug exposure (Broening et al., 2001; Skelton et al., 2006; Sprague et al., 2003; Vorhees et al., 2004; Williams et al., 2003; Able et al., 2006; Skelton et al., 2008, 2009). Also, in addition to learning impairments, there is evidence that MDMA exposure may impede the ability of subjects to adapt their behavior to changing consequences. For example the Wisconsin Card Sorting Task (WCST) utilizes a constant changing of task rules that is used to access cognitive flexibility and it has been found that Ecstasy users are impaired on this task (Smith et al., 2006; VonGeusau et al., 2004). Similarly tasks that assess associative learning have been found to be impaired due to perseverative responding in Ecstasy users (Montgomery et al., 2005) and Ecstasy use has also been associated with task switching deficits using a modified Stroop task (Lamers, Bechara, Rizzo, & Ramaekers, 2006; Dafters, 2008). Within the animal literature there is also evidence that reversal learning is impaired after MDMA exposure where MDMA treated animals have shown deficits during reversal phases of the Morris water maze (Skelton et al., 2006; Skelton et al., 2008, 2009; Williams et al., 2003) where after initial task acquisition the position of the platform is moved (Morris, 1984).

Another issue of interest in MDMA research is whether Ecstasy exposure results in drug tolerance versus sensitization. Ecstasy users have reported having to increase the amount of the drug they take to experience the positive effects of the drug (Parrott, 2001) indicating they become tolerant to the effects of the drug. However, animal studies have found mixed results where some studies have found evidence of drug tolerance occurring after repeated exposure to the drug (Brennan & Schenk, 2006; Frederick & Paule, 1997; Frederick et al., 1995, 1998; LeSage et al., 1993; Marston et al., 1999; Piper, Vu, Safain, Oliver, & Meyer, 2006; Shankaran & Gudelsky, 1999) and others have reported repeated MDMA exposure produces behavioral sensitization (Kalivas, Duffy, & White, 1998; Li, Marek, Vosmer, & Seiden, 1989; Modi, Yang, Swann, & Dafny, 2006; Moyano, Del Rio, & Frechilla, 2005; Spanos & Yamamoto, 1989). The reasons why such conflicting findings as to whether repeated MDMA exposure results in tolerance or sensitization are unclear. However, Brennan and Schenk (2006) suggested that repeatedly administering low doses of MDMA may result in sensitization developing to the effects of MDMA while tolerance may develop following the administration of large chronic or binge doses.

Therefore the present study examined the effect of a binge regime of MDMA on acquisition in the partially baited radial arm. It was hypothesized that MDMA-treated rats would be impaired in their ability to adapt their behavior compared to saline controls which would be evident by MDMA-treated rats acquiring the new task more slowly than controls during acquisition and reversal phases. Acute doses of MDMA (4 mg/kg) and saline were also administered during acquisition and reversal training in order to examine whether behavioral tolerance would be evident in rats that were previously exposed to MDMA.

2. Materials and methods

2.1. Subjects

The subjects were twenty white male Sprague–Dawley rats that were approximately three to 4 months old at the beginning of the study. The rats were bred in-house and were caged individually.

212 They were experimentally naïve at the beginning of the study and
 213 were kept at 85–90% (between 218 and 324 grams) of their free
 214 feeding body weight. The rats began habituation training around
 215 2 weeks after reaching this weight. They had continuous access
 216 to water and were kept on a 12:12-h dark:light cycle and were
 217 run during the dark phase of this cycle.

218 2.2. Apparatus

219 The maze consisted of an aluminum eight arm radial maze that
 220 was secured to a wooden base. The exact measurements and layout
 221 of the maze can be seen in *Kay, Harper, and Hunt (2010)*. Chocolate
 222 chips were used as reinforcers which were placed in circular plastic
 223 Petri dishes that were attached to the ends of the maze arms. Four
 224 open Petri dishes (without lids) were used to house the obtainable
 225 chocolate chips in the reinforced arms. In the non-reinforced arms
 226 four other Petri dishes with lids that had several small holes drilled
 227 in them had chocolate chips sealed inside of them. This meant that
 228 the rats could not obtain them but allowed the odor of the choco-
 229 late chips to permeate from the dishes preventing the rats from
 230 solving the task using the smell of the chocolate (for more detail
 231 see *Kay et al., 2010*). Chocolate chips in the Petri dishes with lids
 232 were replaced daily. The maze was also wiped out each day to re-
 233 move sawdust and other debris. A digital stopwatch was used to
 234 record the amount of time it took a rat to complete a trial.

235 2.3. Behavioral procedure

236 To habituate the rats to the new environment of the maze, they
 237 were individually placed inside the centre hub of the maze and
 238 were allowed to move around freely. On the first day chocolate
 239 chips were placed in the centre of the maze and three chips were
 240 placed in each of the arms. One chocolate chip was placed near
 241 the opening of the arm, another half way down the arm and one
 242 in an open Petri dish at the end of the arm. Rats were given
 243 10 min (or until all chips were consumed) to explore the maze.
 244 On the second day of habituation rats were placed in the centre
 245 of the maze and allowed to explore for seven and a half minutes
 246 (or until all chips were consumed). This time chocolate chips were
 247 placed in the middle of the maze and one chip was placed in the
 248 centre of each arm while another was placed in the Petri dish at
 249 the end of each arm. On the third day of habituation the rats were
 250 placed in the maze for 5 min (or until all the chips were eaten) with
 251 one chocolate chip placed in the middle of the maze and one chip
 252 in the Petri dish at the end of each arm. During the habituation
 253 days the arms the rats visited were recorded to ensure they were
 254 visiting all arms of the maze.

255 The day after completing the habituation the rats were ran-
 256 domly assigned into either the experimental binge MDMA group
 257 or the saline control group (10 rats in each group). Rats were given
 258 four intraperitoneal injections of MDMA (10 mg/kg) or saline
 259 (0.9%) at 2 h intervals on a single day. The following day rats were
 260 given a rest day. Training began after the rest day (2 days after the
 261 injections). The four reinforcer arms were randomly selected for
 262 each rat with no more than two consecutive arms were used for
 263 each rat and each arm was used approximately the same number
 264 of times between rats. Petri dishes without lids were placed in
 265 the reinforcer arms and two chocolate chips were placed in the
 266 bottom of the dish. Petri dishes with lids, that contained two choc-
 267 olate chips sealed inside it, were placed in the four remaining non-
 268 reinforced arms. At the beginning of a trial rats were placed in the
 269 centre of the maze with their head facing in the direction of arm
 270 number one. The rats were then allowed to enter four arms after
 271 which time they were removed from the maze. A choice or arm en-
 272 try was defined as all four feet passing the line formed between the
 273 wood of the centre of the maze and the metal at the beginning of

the arm of the maze. These four arm entries constituted a single
 trial. After a trial was completed and the rat was removed the maze
 was then re-baited and the rat was placed back in the maze. A set
 inter-trial interval was not used it was simply the time taken to re-
 bait the maze and retrieve the rat from the cage which took
 approximately 15–20 s. Each rat received three trials in succession
 per day. Each group of rats was given five sessions of training per
 week.

The binge and control rats had the same sets of reinforced and
 non-reinforced arms, so that the first rat in both groups had the
 same reinforced arms and so that there were matched pairs of rats.
 This was done to control for difficulty of the task in case some sets
 of maze arms were easier to learn than others. The rats were run in
 their matched pairs. This was done as it was more convenient in
 terms of not having to change the arms that needed to be baited
 so often. However, to control for order or odor scenting in the maze
 the running was counterbalanced so that which rat ran first was
 changed on alternate days so 1 day the binge rat in the pair would
 run first and the next day the control rat was run first.

The first part of the study is referred to as the ‘initial acquisition
 phase’ where both groups of rats had to reach a criterion of a group
 average of at least 85% for six consecutive days before they had
 been considered to achieve task acquisition. This took 24 training
 sessions and occurred 30 days after drug treatment. Two days
 (32 days post drug treatment) after the first phase was completed
 the second phase began. This second phase examined the effects of
 acute challenges of MDMA after the rats had acquired the radial
 maze task. During this phase each rat from the previous experi-
 mental and control groups received all drugs and doses. As this
 study was conducted in between the acquisition and reversal
 phases of the previous experiment each rat used its original set
 of four reinforced arms. To ensure that no residual acute drug ef-
 fects were present the final phase of the experiment began 3 days
 after completion of the second phase and hence began 46 days post
 binge drug treatment. During the final phase of the experiment the
 effect of changing the rules of the task was assessed. During this
 third phase each rat’s previously reinforced maze arms now did
 not contain obtainable reinforcers and the previously non-rein-
 forced arms now contained obtainable reinforcers. Rats continued
 to run as in the first phase of the experiment where they were al-
 lowed to enter four arms of the maze per trial and received three
 trials of training per day. As in the first phase rats were run in their
 matched pairs and the order in which the rats within the pairing
 ran was counterbalanced as before. Training continued until a
 90% level of accuracy for both groups was achieved. During this
 reversal phase this took eighteen training sessions which con-
 cluded 70 days post binge drug treatment.

274 2.4. Pharmacological procedure

275 Drugs used were saline 0.9% and MDMA 10 mg/kg (four injec-
 276 tions via i.p. injection – one injection every 2 h) which were pre-
 277 pared on the day of use by dissolving to the required dose in
 278 0.9% saline solution. Unfortunately one of the binge rats died after
 279 receiving the binge regime of MDMA and therefore, there were
 280 only nine rats in the binge group, compared to ten in the saline
 281 control group. During the acute drug administration phase of the
 282 experiment MDMA 4.0 mg/kg was used and it was also dissolved
 283 to the required dose in 0.9% saline solution. This dose was selected
 284 as it has been found to produce significant effects on partially bai-
 285 ted radial maze performance (*Kay et al., 2010*). Each rat was in-
 286 jected i.p. 20 min before running and each drug dose was re-
 287 peated; therefore two doses of 4 mg/kg MDMA and 0.9% saline
 288 were administered to each rat. At least 1 day was left in between
 289 drug treatments to control for carry on effects of the drugs. On

these days the rats were trained in the maze without being administered drugs.

2.5. Performance measures

Arm entries were recorded in the order in which they occurred and error type was also recorded. A working memory error was defined as re-entering an arm already visited during a trial and a reference memory error was defined as entering an arm that had never contained reinforcers. If during a trial a rat re-entered a non-reinforced arm, the first instance was recorded as a reference memory error, while the second visit was recorded as a working memory error. Overall average percent correct accuracy for each individual was calculated by combining all errors made (irrespective of whether they were working or reference memory errors). Individual averages were then combined together to obtain a group average. The time it took to complete a trial in seconds was also recorded. Timing commenced from letting the rat go in the centre of the maze, till when all four feet had passed over the entrance of the fourth arm the rat entered. Trial completion time was included as a measure to pick up more sensitive differences in patterns of responding if there were no discernable differences in error type. Mean trial completion times, in seconds, were calculated for each rat by averaging the three completion times from each trial together for a given session. These values were then combined in order to calculate a group mean for trial completion time. To examine the number and patterns of errors produced, the number of working memory and reference memory errors made per session for each rat was recorded. Working memory errors made per session for each rat was calculated by summing the number of working memory errors made in the three trials. Reference memory errors per session for each rat were also obtained by adding together the number of reference memory errors made across the three trials. These calculations were then converted into percentage error values by taking the mean number of working memory errors and dividing by nine (the total number of working memory errors possible in a session). Mean reference memory errors were divided by twelve (the number of possible reference memory errors in a session). These figures were then multiplied

by one hundred. Note presenting working and reference memory as percentages took into account the fact that a rat could not make as many working memory errors as reference memory errors in a session (i.e., a working memory error can only occur after a reinforced arm has already been appropriately visited, whereas a reference memory error can occur at any time). All inferential statistics were calculated using an alpha level of 0.05 and all *p*-values are given to two decimal places. In all figures error bars show standard error of the mean.

3. Results

During task acquisition both the binge treated MDMA and saline control group initially produced a similar level of accuracy but the saline group's performance improved at a faster rate than the binge MDMA group. This trend was confirmed by a two-way mixed ANOVA that found a significant interaction between session and group, $F(23, 391) = 2.90, p < 0.05 (p < 0.01)$. There were also main effects for session, $F(23, 391) = 104.89, p < 0.05 (p < 0.01)$ and group, $F(1, 17) = 16.23, p < 0.05 (p < 0.01)$. During the reversal phase both groups produced a similar inaccurate level of performance that improved over the training sessions. However the control group's performance improved at a faster rate than that of the MDMA treated group with a significant interaction between session and group $F(17, 289) = 4.52, p < 0.05 (p < 0.01)$. There were also main effects for session, $F(17, 289) = 184.86, p < 0.05 (p < 0.01)$, and group, $F(1, 17) = 31.81, p < 0.05 (p < 0.01)$.

Trial completion times for both groups during the initial acquisition phase of the experiment decreased as training continued (see Fig. 2) which were confirmed by a main effect for session, $F(23, 391) = 26.02, p < 0.05 (p < 0.01)$. It also depicts that trial completion times between the two groups were similar, although the binge group did tend to complete trials more quickly during the first few sessions of training. A two-way mixed ANOVA revealed a significant interaction between group and session, $F(23, 391) = 1.69, p < 0.05 (p = 0.03)$ and no main effect for group, $F(1, 17) = 0.76, p > 0.05 (p = 0.40)$. Despite showing decreases in accuracy during initial learning as seen in Fig. 1 there did not seem

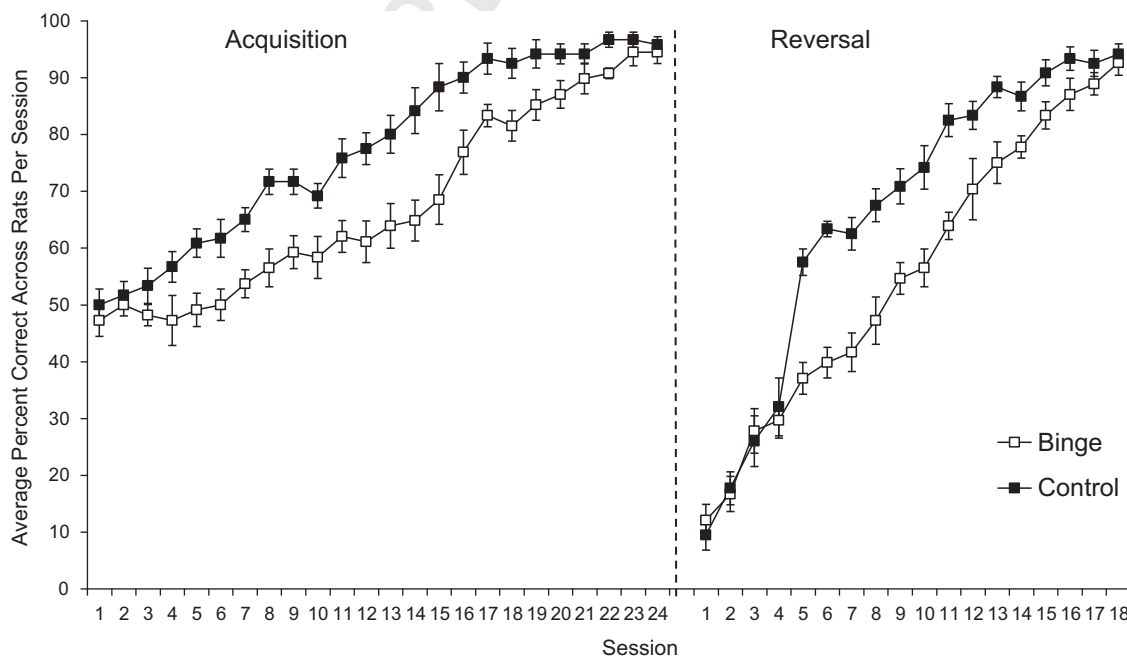


Fig. 1. Average percent correct per session across all rats in both the binge MDMA group and the saline control group for acquisition and reversal training.

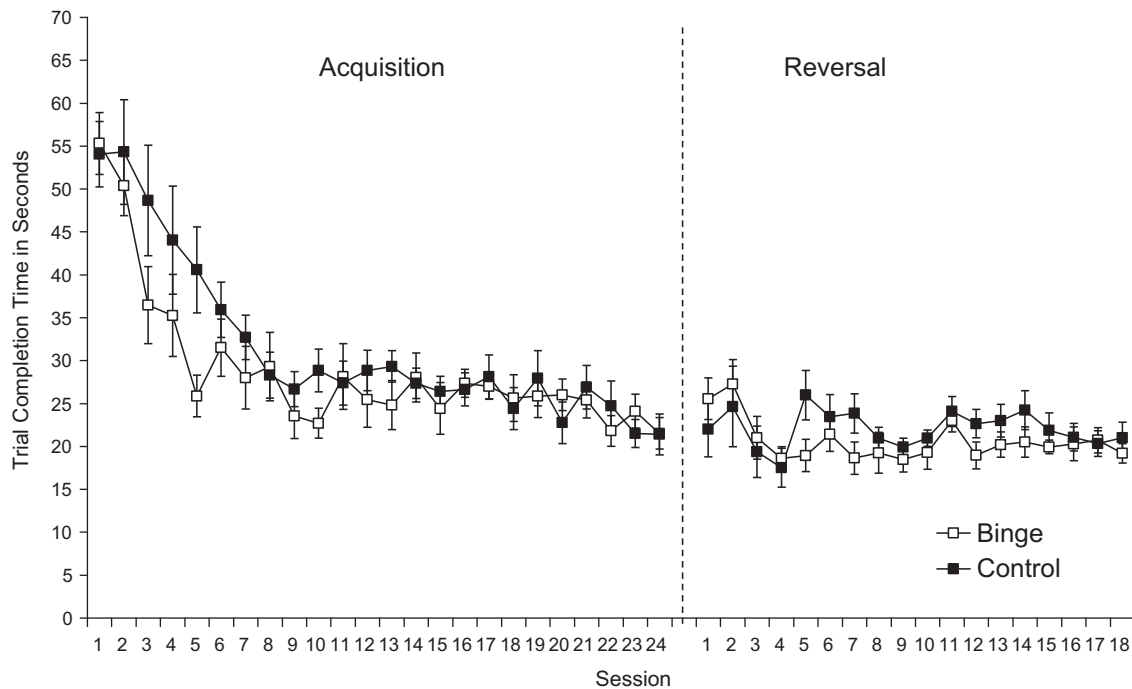


Fig. 2. Average trial completion time in seconds per session across all rats in the binge MDMA and the saline control group for acquisition and reversal training.

to be any obvious effect of changing the task on trial completion time as shown in Fig. 2. There also does not seem to be any difference in trial completion times between the two groups during the reversal phase. These effects were supported as no significant interaction between group and session was found, $F(17, 289) = 0.64$, $p > 0.05$ ($p = 0.86$). There was also no main effect for group, $F(1, 17) = 1.24$, $p > 0.05$ ($p = 0.28$). The ANOVA revealed a main effect for session, $F(17, 289) = 3.78$, $p < 0.05$ ($p < 0.01$) but there was no consistent trend in the data over sessions.

To analyze the error data a two-way repeated measures ANOVA was conducted on the acquisition data comparing error type and training. During the acquisition phase a significant interaction was found between error type and session, $F(23, 414) = 35.09$, $p < 0.05$ ($p < 0.01$) indicating a significant difference in the type of errors made across the training sessions during task acquisition. During the reversal phase a significant interaction between error type and session was also found, $F(17, 306) = 95.30$, $p < 0.05$ ($p < 0.01$). When comparing the working and reference memory data on Fig. 3 it is obvious that more reference memory errors were made than working memory errors during both acquisition and reversal phases of the study. These effects were examined further by individually analyzing the data for working and reference memory errors across training sessions and between groups.

Very few working memory errors were made during acquisition (see Fig. 3). The saline control group tended to make more working memory errors than the MDMA binge group during the first three training sessions but on subsequent training sessions there appeared no obvious group differences or trends in the data. A two-way mixed ANOVA revealed a significant interaction between session and group, $F(23, 391) = 2.46$, $p < 0.05$ ($p < 0.01$). Subsequent post hoc t -tests showed that only on session 3 was there a significant difference between the two groups ($t(17) = 3.0$, $p < 0.01$). During acquisition there was no main effect for session, $F(23, 391) = 0.92$, $p > 0.05$ ($p = 0.58$) and no main effect for group, $F(1, 17) = 0.01$, $p > 0.05$ ($p = 0.93$). During the reversal phase of the study both groups made very few working memory errors with no clear differences between the two groups. Also the number of working memory errors remained fairly stable across the entire

reversal phase and there was no significant interaction between session and group, $F(17, 289) = 0.63$, $p > 0.05$ ($p = 0.87$). There were also no main effects for session, $F(17, 289) = 0.83$, $p > 0.05$ ($p = 0.65$) and group, $F(1, 17) = 1.80$, $p > 0.05$ ($p = 0.20$).

During the acquisition phase both groups made a large number of reference memory errors at the beginning of training and this steadily decreased over the training sessions as the task was acquired (see Fig. 3). During the acquisition phase the saline control rats appeared to reduce the number of reference memory errors made across sessions at a faster rate than the binge MDMA-treated rats. These effects were corroborated by a two-way mixed ANOVA that found main effects for session, $F(23, 391) = 84.91$, $p < 0.05$ ($p < 0.01$) and group, $F(1, 17) = 17.84$, $p < 0.05$ ($p < 0.01$) which were moderated by a significant interaction between group and training session, $F(23, 391) = 2.20$, $p < 0.05$ ($p < 0.01$). During the reversal phase both groups made a very high number of reference memory errors during the first couple of sessions but then the number of reference memory errors made by the control rats decreased at a faster rate than the MDMA-treated rats producing a significant interaction between group and training, $F(17, 289) = 4.13$, $p < 0.05$ ($p < 0.01$). During the reversal phase there was also a main effect for session, $F(17, 289) = 171.76$, $p < 0.05$ ($p < 0.01$) and a main effect for group, $F(1, 17) = 29.64$, $p < 0.05$ ($p < 0.01$).

During the acute drug treatment phase both the binge MDMA group and the saline controls produced a very high level of accuracy during acute saline administration with no obvious difference between the two groups (see Fig. 4). With acute MDMA administration both groups showed a marked impairment where the saline control group appeared to be more affected by the acute administration of MDMA than the binge MDMA group. This effect was supported by a 2-way mixed ANOVA with a significant interaction between group and drug treatment, $F(1, 17) = 9.72$, $p < 0.05$ ($p < 0.01$). There was also a main effect for acute drug treatment, $F(1, 17) = 213.33$, $p < 0.05$ ($p < 0.01$) but no main effect for group, $F(1, 17) = 3.62$, $p > 0.05$ ($p = 0.07$).

Both the binge MDMA and saline control groups produced similar short trial completion times during the saline administration sessions (see Fig. 5). During acute MDMA administration both

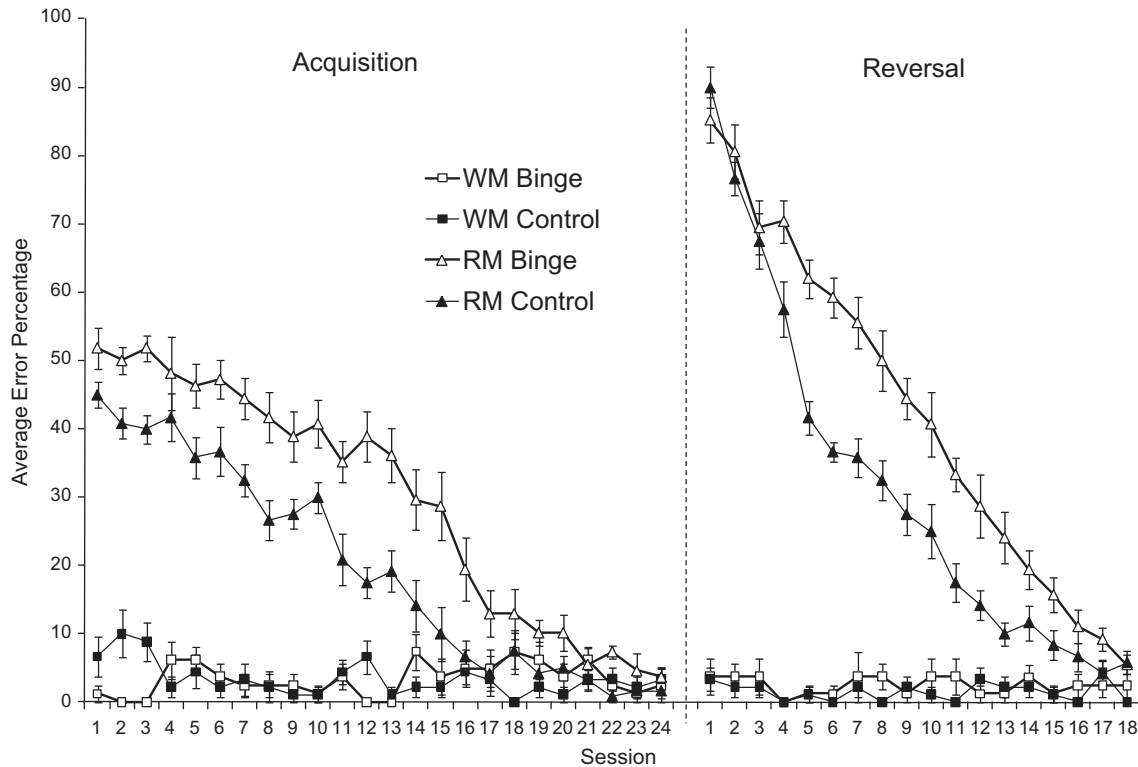


Fig. 3. Average working and reference memory error percentages across all rats in both the binge MDMA and saline control groups during acquisition and reversal training.

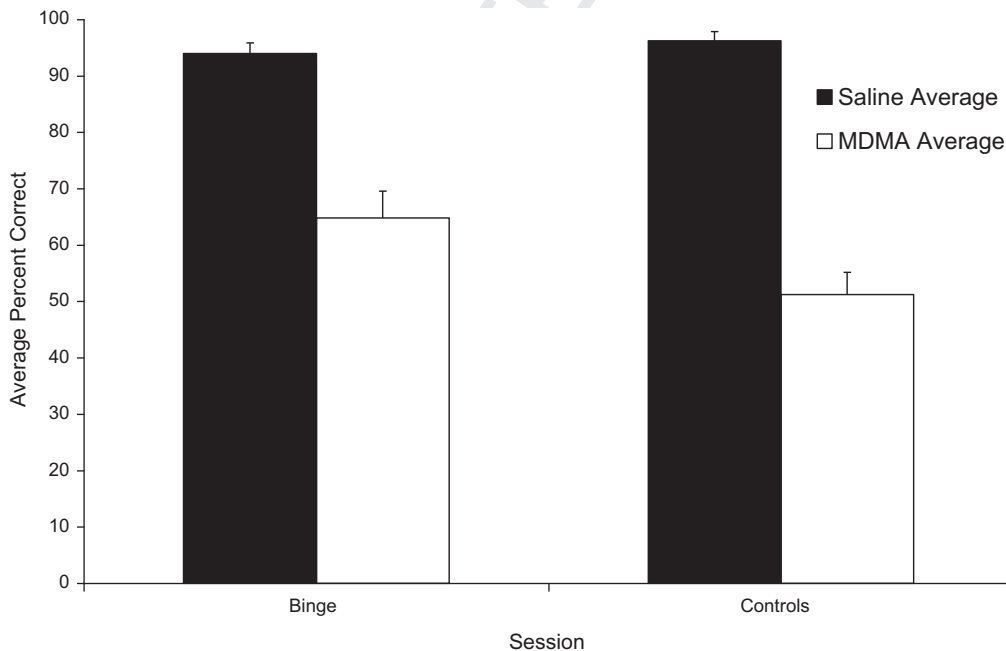


Fig. 4. Average percent correct across all rats in both the binge MDMA group and the saline control group for the acute doses of saline and MDMA.

486 groups produced much longer trial completion times with no obvious
 487 differences between the groups. There was no significant interaction
 488 between acute drug treatment and group, $F(1, 17) = 1.26$,
 489 $p > 0.05$ ($p = 0.28$) and no main effect for group, $F(1, 17) = 1.38$,
 490 $p > 0.05$ ($p = 0.26$). There was a main effect for drug treatment,
 491 $F(1, 17) = 108.65$, $p < 0.05$ ($p < 0.01$) and from Fig. 5 it is obvious

492 that acute MDMA treatment impaired performance more than
 493 acute saline treatment.
 494 Acute saline administration produced very few working memory
 495 errors in either group while acute MDMA exposure produced
 496 a slight increase in working memory errors with the binge MDMA
 497 group producing marginally more working memory errors than the

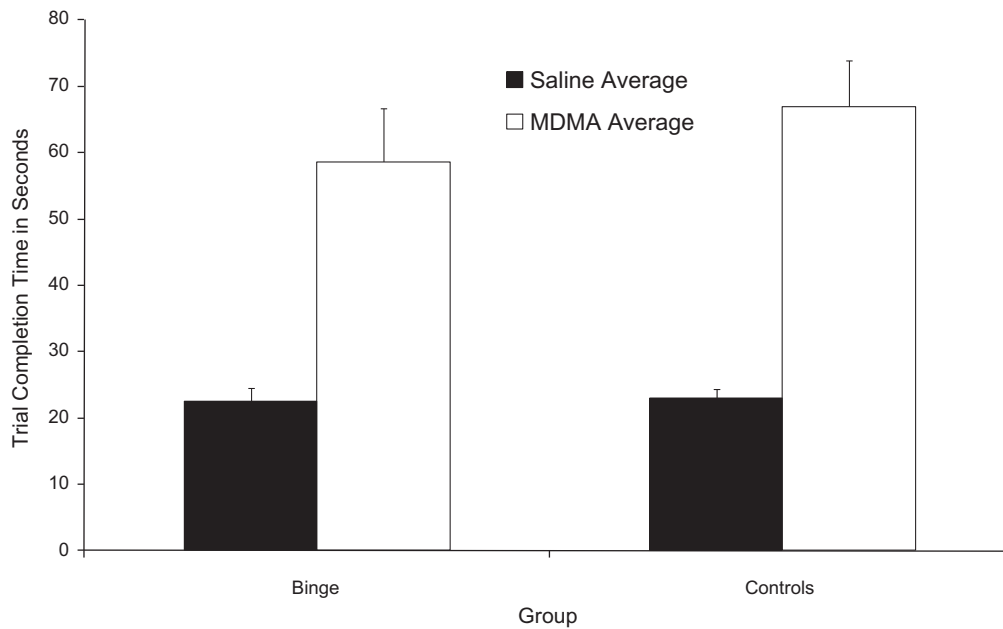


Fig. 5. Average trial completion time in seconds across all rats in both the binge MDMA group and the saline control group for the acute doses of saline and MDMA.

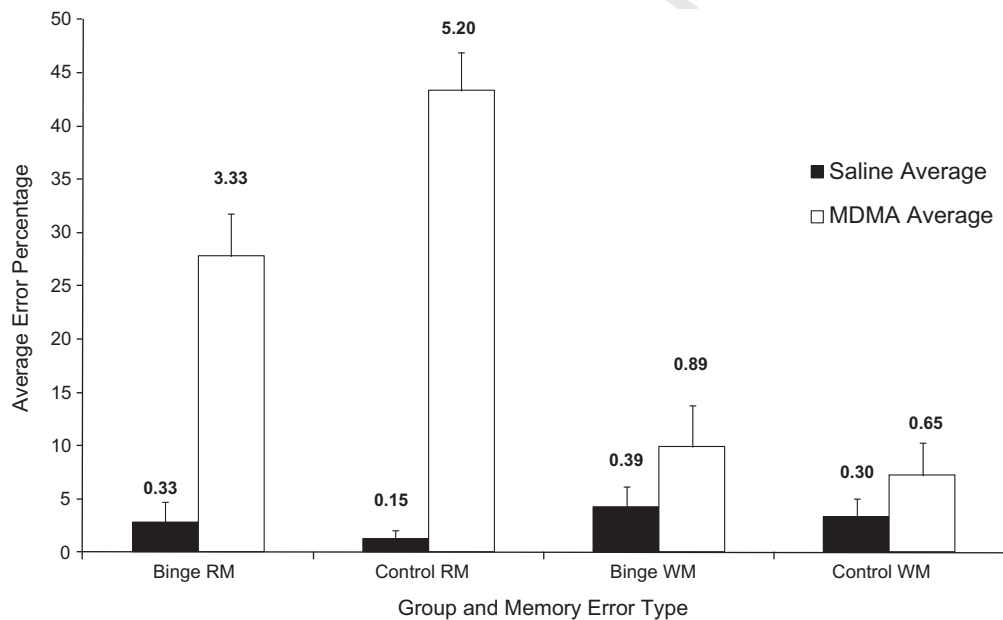


Fig. 6. Average error percentage of working memory (WM) and reference memory (RM) errors across all rats for both the MDMA binge group and the saline control group. The values given above each bar are the mean number of total working memory or reference errors made in each condition across rats.

saline control group (see Fig. 6). These small effects were shown to be non significant with no interaction between acute drug treatment and group, $F(1, 17) = 0.13, p > 0.05 (p = 0.72)$ and no main effects for acute drug treatment, $F(1, 17) = 4.30, p > 0.05 (p = 0.05)$ or group, $F(1, 17) = 0.61, p > 0.05 (p = 0.45)$.

Very few reference memory errors were made by either group with acute saline administration and acute MDMA exposure produced a large number of reference memory errors made in both groups (see Fig. 6). The saline control group appeared to make more reference memory errors than the binge MDMA group when administered acute doses of MDMA which was confirmed by a significant interaction between acute drug treatment and group,

$F(1, 17) = 19.11, p < 0.05 (p < 0.01)$. There were also main effects for acute drug treatment, $F(1, 17) = 294.46, p < 0.05 (p < 0.01)$ and group, $F(1, 17) = 6.25, p < 0.05 (p = 0.02)$.

To compare error types a 3-way mixed ANOVA was conducted examining memory type (working and reference memory errors), acute drug treatment (MDMA or saline) and group (binge and control). There was a 3-way interaction between memory type, acute drug treatment and group, $F(1, 17) = 9.36, p < 0.05 (p < 0.01)$. Thus the type of error made differed significantly depending on the type of acute drug administered and whether the subjects had been pre-treated with binge MDMA or saline. Referring to Fig. 6 this result suggests there were more reference memory errors than working

memory errors during acute MDMA administration and this was more pronounced in the saline control group.

There was also an interaction between memory type and group, $F(1, 17) = 4.81, p < 0.05 (p = 0.04)$. Thus there was a significant difference in the type of errors made between the two groups. Examining Fig. 6 shows the control group made more reference memory errors than the binge group. In addition there was a significant interaction between memory type and acute drug treatment, $F(1, 17) = 88.42, p < 0.05 (p < 0.01)$. Hence there was a significant difference in the type of memory errors made during acute MDMA and saline treatments where MDMA treatment produced more reference memory than working memory errors. Finally there was a significant interaction between acute drug treatment and group, $F(1, 17) = 6.90, p < 0.05 (p = 0.02)$, indicating a significant difference on the effects of administering acute doses of MDMA and saline on group performance. Main effects for memory type, $F(1, 17) = 39.13, p < 0.05 (p < 0.01)$ and acute drug treatment, $F(1, 17) = 169.96, p < 0.05 (p < 0.01)$. There was no main effect for group, $F(1, 17) = 2.58, p > 0.05 (p = 0.13)$.

4. Discussion

The aim of the current study was to examine if binge MDMA exposure would impair acquisition of the partially-baited radial-arm maze task. During the initial acquisition phase both groups of rats learnt the task with accuracy increasing across training sessions. During this phase the binge MDMA group showed evidence of a learning impairment compared to controls because they acquired the task at a significantly slower rate. However, they were able to eventually acquire the task and perform at a similar level to the saline controls. Trial completion time significantly decreased throughout the acquisition for both groups. There were no significant differences between the two groups which suggests that binge MDMA exposure did not affect this aspect of performance. In terms of the types of error made, during acquisition both groups produced a similar low number of working memory errors but both groups produced a high number of reference memory errors initially, but the rate at which reference memory errors decreased was slower in the MDMA treated group. Therefore MDMA exposure produced evidence of a learning impairment and this cognitive deficit appeared to specifically involve an impairment to reference memory processes.

This finding is consistent with previous literature that has utilized Morris and Cincinnati water maze tasks that have found that chronic or binge MDMA exposure impairs the acquisition of tasks requiring reference memory processes (Broening et al., 2001; Robinson et al., 1993; Skelton et al., 2006; Sprague et al., 2003; Vorhees et al., 2004; Williams et al., 2003; Able et al., 2006; Skelton et al., 2008, 2009). However these results appear somewhat inconsistent with evidence from automated operant-based tasks (such as DMTS) that have failed to find evidence of learning impairments (e.g., Li et al., 1988; Byrne, Baker, & Poling, 2000; Moyano et al., 2005; Winsauer et al., 2002). A possible reason why some studies have failed to show evidence of an ongoing impairment following MDMA administration could be due to the tasks not having a significantly demanding reference memory component compared to the maze tasks that have found evidence of MDMA-induced cognitive impairments. Another possible explanation for the conflicting findings between operant chamber tasks and maze paradigms is that the latter involves greater spatial memory processes which may be more susceptible to the effects of MDMA exposure. In addition some of these studies used the acquisition of a differential-rate-of-low-reinforcement (DRL) task to assess learning processes (Li et al., 1988; Byrne et al., 2000). It could be argued that these are not complex tasks and do not really assess cognitive function

as they involve simply learning to press a lever at a specific rate and do not require memory processes to the same extent as DMTS or maze type tasks.

The current study also examined if a binge dose of MDMA would affect the ability to adapt to changing consequences when the rules of the radial arm maze paradigm were reversed. During the reversal phase the saline controls learnt the task significantly faster than the MDMA treated group. Thus MDMA exposure significantly impaired the ability of the rats to adapt their behavior to a change in task demands. However by the end of training both groups produced a similar level of performance indicating that eventually the MDMA group was able to acquire the new task. Also in this phase there was no real increase in trial completion time for both groups and this did not differ between the two groups. When examining the kind of errors made both groups produced very few working memory errors and this did not differ significantly between the two groups. In contrast the reversal phase produced a large increase in reference memory errors for both groups. During this phase the MDMA treated group produced significantly more reference memory errors than the saline controls. Hence once again the impairment produced by MDMA exposure involved reference memory processes. However, it should be noted that while entering non-reinforced arms in this phase was counted as a reference memory error the same way as in the previous phase the cognitive process they were assessing may be different. This is because reference memory errors in the reversal phase meant subjects were failing to alter their behavior rather than remembering previously learnt information about which arms contained reinforcement. Thus, the reference memory errors made in this phase can be thought of as perseverative errors which is a pattern of impairment that has been seen in previous research examining the acute effects of MDMA exposure (Frederick et al., 1995; Verrico et al., 2008) and is a pattern of responding that has been found in Ecstasy users (Montgomery et al., 2005; Smith et al., 2006; VonGeusau et al., 2004; Dafters, 2008; Verrico et al., 2008).

The finding that binge MDMA exposure produced significant impairments in cognitive flexibility is consistent with previous literature (Montgomery et al., 2005; Smith et al., 2006; VonGeusau et al., 2004; Dafters, 2008; Lamers et al., 2006) that has found that Ecstasy users are impaired at tasks that involve altering their behavior in response to changing task demands. In addition it is consistent with research (Able, Gudelsky, Vorhees, & Williams, 2005; Skelton et al., 2006, 2008, 2009; Williams et al., 2003) that has utilized reversal phases in the Morris water maze which require subjects to alter their behavior once the initial task had been acquired. There is also some neurochemical evidence within the literature to further support these findings whereby monkeys produce significant perseverative impairments on reversal discrimination tasks following experimental 5-HT depletion in the prefrontal cortex (Clarke, Dalley, Crofts, Robbins, & Roberts, 2004; Clarke, Walker, Dalley, Robbins, & Roberts, 2007). As the binge regimen of MDMA utilized within the current study has been shown to significantly reduce 5-HT levels in multiple brain regions including the prefrontal cortex (Scanzello, Hatzidimitriou, Martello, Katz, & Ricaurte, 1993) this may account for the finding that MDMA exposure impairs cognitive flexibility. However as this study did not conduct any physiological measures of 5-HT activity it is unknown as to the degree of 5-HT depletion subjects in the current study experienced.

The deficits seen in the current study appeared to be relatively long-term as the subjects continued to show impairment during the reversal phase of the task which began 46 days after drug treatment and did not reach a similar level of performance to controls till around 68 days post drug treatment. Therefore while performance of the MDMA treated animals appeared to be similar to the saline controls by the time acquisition training was finished,

the fact they then showed impairment when they were required to alter their behavior suggests they still were suffering from an underlying cognitive impairment. Hence one possible reason why some research has failed to show evidence of cognitive impairments produced by MDMA exposure is that they have involved tasks where the subjects have already received a lot of training on the task. The current study would suggest that an impairment would be more visible on tasks that were still being acquired and those that required subjects to alter their behavior. This may explain why some previous research, such as those that have used DMTS-type tasks (Frederick & Paule, 1997; Frederick et al., 1995; LeSage et al., 1993; Taffe et al., 2001) and other operant-based tasks (Winsauer et al., 2002) have failed to show evidence of MDMA induced deficits as in these studies the cognitive task had already been acquired when subjects were exposed to the regimes of MDMA.

There are several potential explanations for the underlying cause of these cognitive impairments. A more sensory-level explanation for the behavior produced by the subjects exposed to MDMA is that the drug simply impairs their ability to detect extra-maze cues which are generally used to solve radial maze tasks (Liao, Lai, & Lin, 2002). However, this explanation seems unlikely because the visual distortions reported by human Ecstasy users are associated with acute use rather than being an ongoing feature post drug exposure. In other words Ecstasy users only tend to experience visual hallucinations while under the influence of the drug (Peroutka, Newman, & Harris, 1988). These sensory distortions have been linked to the increase in 5-HT activity produced by acute MDMA administration (Liechti & Vollenweider, 2000). Rats in the current were not tested under acute drug exposure (except in one condition) and the binge regime of MDMA used here has been shown to produce a decrease in 5-HT activity (Scanzello et al., 1993). Furthermore, during the training and reversal phases there were no significant differences between MDMA-treated rats and controls in terms of how long it took them to complete trials within the radial maze. Hence it seems unlikely that the impairments found after MDMA exposure were the result of drug induced motor impairments. This finding is consistent with the previous research that has found that MDMA has produced reference memory impairments in maze paradigms without affecting swimming ability as assessed by straight channel swimming tasks (Broening et al., 2001; Vorhees et al., 2003; Williams et al., 2003; Able et al., 2006; Skelton et al., 2006, 2009) which are used to assess motor impairments after drug exposure.

The current study also examined whether rats that had already acquired the radial-arm maze task, and had been exposed to a binge dose of MDMA, would show evidence of behavioral sensitization or tolerance when exposed to acute administration of MDMA compared to saline controls. During acute MDMA administration both the binge MDMA and control groups produced a decrease in accuracy but the control group was significantly more impaired than the binge MDMA treated group. (Although no difference between groups was found in terms of trial completion time or working memory errors). The finding that behavioral tolerance with respect to reference memory function occurred is consistent with previous research (Brennan & Schenk, 2006; Frederick & Paule, 1997; Frederick et al., 1995, 1998; LeSage et al., 1993; Marston et al., 1999; Piper et al., 2006; Shankaran & Gudelsky, 1999) and the finding that acute MDMA administration affected reference memory more than working memory in the radial arm maze is also consistent with previous research (Kay et al., 2010).

A possible confound in the current study was that the acute drug phase of the experiment was run in between the acquisition and reversal phases. There should be no residual acute effects of the drug during the reversal phase because of the 3 day gap between the last acute MDMA drug session and the beginning of

the reversal phase. However, there is the possibility that the acute dose of MDMA may have in some way affected performance during the reversal phase of the task. For example, it is unknown what effect two additional acute doses of MDMA would have on the rats that had already received a binge regime of MDMA. While there is evidence that there is both behavioral (Brennan & Schenk, 2006) and neurochemical (Scanzello et al., 1993) recovery of function after the binge regime of MDMA used in the current study, it is unknown what effect additional low doses of acute MDMA would have on this process. Therefore, there is the possibility that these additional acute doses of MDMA may have affected performance in the reversal phase. This issue might be worth specifically addressing in the future, however it should be noted that the binge group showed fewer reference memory errors than controls when subsequently given acute MDMA. But, when both groups were drug free and subsequently trained in the reversal phase the binge group showed more reference memory errors than controls. Thus if there was a contamination of the acute MDMA exposure on subsequent reversal learning the acute effects, if anything, would have worked against the subsequent memory impairments shown by the binge group in the current study.

The binge regime used in the current study (4×10 mg/kg for 1 day) generally involved less MDMA than much of the previous work that has examined the effects chronic and binge MDMA exposure on cognition. The most commonly used (Broening et al., 2001; Skelton et al., 2006; Vorhees et al., 2004; Williams et al., 2003) chronic regime in the developmental studies involves 20 mg/kg given twice a day for 10 days. Even short course binge regimes have used larger doses of MDMA than the current study. For example Robinson et al. (1993) gave double the injections of 10 mg/kg of MDMA to that used in the current study and both Able et al. (2006) and Skelton et al. (2008) used four injections of 15 mg/kg in a day. While exposing subjects to a smaller amount of MDMA may result in a less obvious impairment in performance the regime used in the current study has been shown to produce 5-HT damage (Scanzello et al., 1993) and result in significant behavioral effects (Brennan & Schenk, 2006). In addition there has been criticism that the doses of MDMA previously used in chronic studies are unrealistically large compared to human use of the drug (Baumann, Wang, & Rothman, 2007). Hence the finding that the current study was able to show evidence of cognitive deficits while administering a smaller regime of MDMA adds to the existing MDMA literature.

To summarize, the current study found when rats were treated with a binge regime of MDMA and began training shortly after drug exposure they took significantly longer and had a slower rate of learning compared to saline controls when acquiring the partially baited radial arm maze. As subjects were able to eventually acquire the task and perform at a similar level to the controls it appeared this impairment was transient. However, when the rules of the task were changed the MDMA-treated rats were significantly slower to adjust their behavior and learn to perform the new task. Therefore, this learning impairment appeared to be long-term in nature as it continued to impair performance up to 68 days post drug treatment. Evidence of behavioral tolerance was found as rats that had not experienced previous exposure to MDMA were more impaired when administered acute challenges of MDMA. Finally the impairments produced by MDMA exposure, both from the binge regime and acute drug treatments, involved reference memory processes more than working memory processes. The underlying nature of this impairment remains unclear but it may be due to a long-term memory deficit, an impairment in understanding task rules or a perseverative pattern of responding. These findings imply human Ecstasy users may show deficits in acquiring information and may experience deficits in cognitive flexibility.

5. Uncited references

Cottler, Womack, Compton, and Ben-Abdallah (2001), Dafters, Hoshi, and Talbot (2004) and Parrott, Sisk, and Turner (2000).

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