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Gestational treatment with methylazoxymethanol (MAM) that disrupts hippocampal-dependent memory does not alter behavioural response to cocaine

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ABSTRACT

Schizophrenia is associated with increased rates of substance abuse that are thought to be the result of changes in cortical and mesolimbic dopamine activity. Previous work has shown that gestational methylazoxymethanol acetate (MAM) treatment induces increased mesolimbic dopamine activity when given around the time of embryonic day 17 (ED17), suggesting that MAM treatment may model some aspects of schizophrenia. Given that increased dopaminergic activity facilitates aspects of drug self-administration and reinstatement of drug seeking, the current experiments sought to assess cocaine self-administration in MAM treated animals. Experiment 1 examined the acquisition of cocaine self-administration in ED17 MAM and saline treated rats using a sub-threshold dose of cocaine. In experiment 2 ED17 MAM and saline treated animals were trained to self-administer cocaine and were then assessed under varying doses of cocaine (dose–response), followed by extinction and drug-induced reinstatement of responding. A subset of these animals was trained on a win-shift radial maze task, designed to detect impairments in hippocampal-dependent memory. In experiment 3, MAM and saline treated animals were assessed on a progressive ratio schedule of cocaine delivery. Finally, in experiment 4 MAM and saline treated animals were assessed on cocaine-induced locomotor activity across a range of doses of cocaine. MAM treatment disrupted performance of the win-shift task but did not alter cocaine self-administration or cocaine-induced locomotion. Implications of these results for the MAM model of schizophrenia are discussed.

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1. Introduction

The notion that schizophrenia is caused by changes in mesolimbic dopamine function has a long history (Davis et al., 1991; van Rossum, 1966). A prominent role for dopamine in the pathophysiology of schizophrenia is supported by the fact that antipsychotic drugs mainly target dopamine D2 receptors (Kapur and Remington, 2001; Seeman et al., 1976), that schizophrenic patients show increased mesolimbic dopamine release in response to amphetamine challenge (Laruelle et al., 1999; Laruelle et al., 1996), and by the finding that long-term psychostimulant use can induce schizophrenia-like symptoms (Curran et al., 2004). The dopamine hypothesis is limited in that some features of schizophrenia, such as cognitive deficits or neuropathology, may not be completely reducible to changes in dopamine activity and function. As such, recent attention has shifted towards neurodevelopmental models of schizophrenia (see for example Lillrank et al. (1995)), and evidence suggests that these models may be able to explain both the neuropathological changes and the hyperdopaminergia associated with schizophrenia (Lipska et al., 2003).

Gestational methylazoxymethanol acetate (MAM) treatment is a neurodevelopmental model of schizophrenia. This mitotoxin induces behavioural and neuropathological changes that resemble those seen in schizophrenia (Moore et al., 2006). Treatment of pregnant rats on embryonic day 17 (ED17) with MAM simulates in the offspring some of the cognitive deficits observed in schizophrenia, including impaired working memory (Gourevitch et al., 2004), attentional set-shifting (Featherstone et al., 2007), behavioural flexibility (Flagstad et al., 2005; Moore et al., 2006) and recognition memory (Flagstad et al., 2005). These behaviours were accompanied by neuropathological changes including reductions in size of the prefrontal cortex (PFC) and hippocampus (Flagstad et al., 2004; Moore et al., 2006), increases in PFC neuronal density (Moore et al., 2006) and alterations in prefrontal responses to dopamine (Flagstad et al., 2004), similar to those changes observed in schizophrenia. Additionally, MAM treated animals show elevated levels of amphetamine-induced locomotion in response to an

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amphetamine challenge (Flagstad et al., 2004; Moore et al., 2006), as well as enhanced dopamine release in the nucleus accumbens to both systemic and localized amphetamine administration (Flagstad et al., 2004). This suggests that MAM treatment results in enhanced activity in the mesolimbic dopamine system.

The pattern of increased mesolimbic dopamine activity coupled with alterations in hippocampal and prefrontal activity in MAM treated animals is broadly consistent with that found in schizophrenia and with the types of change thought to underlie drug abuse in schizophrenia (Chambers et al., 2001). Individuals with schizophrenia show increased rates of drug abuse relative to the general population (Hambrecht and Hafner, 1996; Regier et al., 1990), and it has been argued that this relationship stems from a shared neuropathology common to both substance abuse and schizophrenia (Chambers et al., 2001). For example, heightened dopamine activity within the mesolimbic dopamine pathway plays a strong role in mediating the reinforcing properties of various drugs of abuse (Di Ciano and Everitt, 2004; McFarland and Kalivas, 2001; Roberts and Koob, 1982; Zito et al., 1985). Manipulations which enhance dopamine activity in the mesolimbic dopamine system facilitate the acquisition of drug self-administration (Schenk and Partridge, 1997; Vezina, 2004).

Given that MAM induces neuropathological and neurochemical changes similar to those seen in schizophrenia, and given that both schizophrenia and drug abuse appear to share a similar underlying biology, we predicted that MAM treated animals would show behavioral changes indicative of increased susceptibility to the development substance abuse disorders (i.e. a change in sensitivity to the rewarding properties of cocaine). The present study tested this hypothesis in a number of ways. Specifically, we examined whether MAM treatment altered acquisition of cocaine self-administration for a sub-threshold unit infusion dose, shifted the dose–response function for cocaine on both a fixed ratio (FR) and progressive ratio (PR) schedule, altered the ability of cocaine to reinstate extinguished drug-seeking behaviour, or altered the locomotor stimulant effects of cocaine. As a positive control we determined whether MAM treatment altered hippocampal-dependent learning and memory on a win-shift maze test.

2. Method

2.1. Subjects

Timed pregnant Sprague–Dawley rats were obtained from Charles Rivers (Saint-Constant, Quebec) on day 12 of gestation. On ED17 (day 0 defined as the day of conception) rats were treated with saline or methylazoxymethanol acetate (22 mg/kg, in a volume of 5 mg/ml; Midwest Research Institute, Kansas City, Missouri) administered via an intraperitoneal (IP) injection. For experiment 1, three mothers were injected with saline and seven mothers were injected with MAM. For experiment 2, ten mothers were injected with MAM and six mothers were injected with saline. For experiment 3 and 4, seven mothers were injected with MAM and seven mothers were injected with saline. One hundred and three animals were used (experiment 1, MAM = 13 and saline = 11; experiment 2, MAM = 20 and saline = 14; experiment 3, MAM = 12 and saline = 9; experiment 4, MAM = 12 and saline = 12). Pups were weaned at 28 days of age and housed with a sibling in a clear plastic cage [48 cm × 27 cm × 20 cm]. At this time animals were sexed and females were removed from the study. Prior to testing (around 80 days of age), animals were singly housed. Rooms were maintained on a 12 h light schedule (08:00–20:00). All procedures were carried out in accordance with the Canadian Council on Animal Care and the CAMH animal care committee.

2.2. Surgery

Between 70 and 80 days of age rats underwent surgery to implant a catheter into the right jugular vein. Catheters were constructed from two pieces of Silastic tubing (outer diameter 0.025 and 0.045 in.) connected to a 22-gauge stainless steel tube. The tubing was cemented within a nylon bolt that was anchored to a square piece of Marlex mesh. Heat shrink tubing connected the two pieces of Silastic tubing. Animals were anesthetized with sodium pentobarbital (Somnotol; 65 mg/kg; experiment 1 and 2) or ketamine/xylazine (75 mg/kg and 10 mg/kg respectively; experiment 3) and small incisions were made in the neck and upper back between the shoulder blades. The catheter was inserted into the jugular vein and threaded subcutaneously with the terminal end exiting at the mid-scapular level. The incisions were closed with sutures. Following surgery, animals were given Penlong (1 ml/kg) or Derapen (0.1 mg) to prevent infection. Additionally, to maintain patency, catheters were flushed with 0.05–0.1 ml of a saline solution containing heparin (0.1 ml of 30 IU/ml) every day. Catheter patency was assessed by infusing methohexital (0.1 ml of a 10% solution).

2.3. Apparatus

2.3.1. Cocaine self-administration

Testing was conducted in 22 operant conditioning chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med. Associates Inc., St Albans, VT., USA). Each chamber contained two response levers 4.5 cm wide and 7 cm above the floor of the chamber, and a stimulus light located 6 cm above each lever. A counterbalanced arm held a fluid swivel above the ceiling of the chamber. The swivel was attached at one end by Tygon tubing to a syringe mounted on a motor driven syringe pump (Razel) located outside the chamber. At the other end of the swivel a length of Tygon tubing, encased in a stainless steel tether, connected the animal's catheter to the syringe via the swivel. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. A PC controlled the apparatus and collected the data.

2.3.2. Cocaine-induced locomotor activity

Locomotor response to amphetamine was assessed in custom-built activity monitoring system. This system consisted of sixteen transparent, Plexiglas cages (48 cm by 27 cm by 20 cm), each of which was equipped with 6 photobeam cells capable of detecting horizontal movement.

2.3.3. Win-shift radial maze task

Training took place on an 8 arm radial maze constructed from dark grey Plexiglas. Each individual arm (60 cm long and 9 cm wide) was attached to a central octagonal platform, measuring 40 cm across, elevated 60 cm above the floor. A small metal cup was placed at the end of each arm so that the food reward (a half piece of Froot-Loop® cereal) could be hidden from sight.

2.4. Procedure

2.4.1. Experiment 1

One week after surgery rats were tested for spontaneous acquisition of self-administration of a low dose of cocaine (0.1 mg in 0.1 ml saline per IV infusion, delivered over 5 s). This dose was chosen since it falls below those that have been reported to reliably maintain cocaine self-administration (Horger et al., 1990). Each self-administration session began with a single priming infusion of cocaine, with subsequent responses on the left lever delivering an infusion according to a FR1 schedule. Infusions were accompanied by illumination of the left stimulus light. This light remained on for 20 s after the infusion; during this time responses were recorded, but had no programmed consequences. Throughout the session responses on the right lever were also recorded, but had no programmed consequences. Sessions were 2 h in duration and were conducted on 7 consecutive days. Following the last day of testing all rats were

administered a short acting anaesthetic agent, methohexital (0.1 ml of a 10% solution), to test for patency of the jugular catheters. Rats failing to lose muscle tone within 5 s were eliminated from the study. Two MAM treated animals were removed; leaving 11 saline treated and 11 MAM treated animals.

2.4.2. Experiment 2

Rats were first trained to lever press for food (45 mg Noyes pellets). Rats were food restricted (15 g per day) and placed in the operant conditioning chambers where each response on the left lever delivered a food pellet according to a FR1 schedule. Rats were allowed a maximum of 100 pellets per daily 30 min sessions. Any rats failing to obtain 100 pellets by the third day of training were placed in the operant boxes overnight and allowed 300 food pellets delivered according to the FR1 schedule. Using this procedure all rats were responding for 100 pellets within 4 days of training. Thereafter rats were maintained on 20 g of food per day. Animals then underwent surgery to implant IV catheters, as described above. One week after surgery rats were placed in the operant conditioning chambers for a 2 h drug self-administration session. The session began with illumination of the houselight. Responses on the left lever delivered an infusion of 0.25 mg/infusion cocaine IV over 5 s. The infusion was accompanied by illumination of the stimulus light above the lever. This light remained illuminated for 20 s after the infusion during which period responses were recorded but did not have any programmed consequences.

2.4.2.1. Dose–response test. After 12 training sessions responding was stable for 0.25 mg/infusion. Over 4 separate sessions the unit infusion dose was varied such that animals responded during one 2 h session for 0.0625, 0.1, 0.25 and 0.5 mg/infusion doses. Test sessions occurred every other day, according to a Latin square design. On the intervening days, subjects responded for the original dose (0.25 mg/infusion). An additional two sessions with the original dose was given following the dose–response test in order to re-stabilize responding prior to the beginning of extinction. Seventeen MAM treated and 14 saline treated animals completed this phase of testing.

2.4.2.2. Extinction. Extinction training was identical to the original self-administration training with the exception that bar presses activated the pump but did not deliver any infusions. Bar presses also activated the stimulus light such that responding associated with the light cue would also extinguish. This phase lasted until the number of responses was fewer than 20 per session (which took for 9 days).

2.4.2.3. Reinstatement. Following extinction, animals were tested for drug-induced reinstatement of bar press responding. Reinstatement sessions were identical to extinction sessions with the exception that animals were given systemic IP injections of cocaine HCl (10 mg/kg or 20 mg/kg, doses expressed as salt) or saline before each session. Each animal was treated with both doses of cocaine and vehicle according to a Latin squares design. Test sessions occurred every third day, separated by two days of extinction sessions. Fourteen MAM treated and 14 saline treated animals completed reinstatement testing.

2.4.2.4. Win-shift radial maze task. A subset of animals from experiment 2 was tested on the win-shift radial maze task (8 MAM and 8 saline treated animals). No differences were observed between saline and MAM treated animals on any aspect of self-administration performance for this subset of rats. Animals were first restricted to approximately 85% of their pre-experimental body weight before undergoing habituation to the radial maze. Habituation took place over two 10 min sessions. Once habituated to the maze, animals began acquisition training. All 8 arms of the maze were baited with food (1/2 piece of Froot-Loop® cereal) and subjects were allowed 15 min to locate and consume all 8 pieces of food. The primary measure of interest was the number of revisits to previously depleted arms (errors). Training occurred until saline treated animals were able to reach a pre-specified performance criterion ($\leq$1 error per session).

2.4.3. Experiment 3

2.4.3.1. Responding on a progressive ratio schedule. The procedure for experiment 3 followed the same food and FR1 training as outlined in experiment 2 above. Once responding stabilized after 6 training sessions on the FR1 schedule at a dose of 0.25 mg/infusion, a progressive ratio (PR) schedule of reinforcement was introduced. On this schedule the

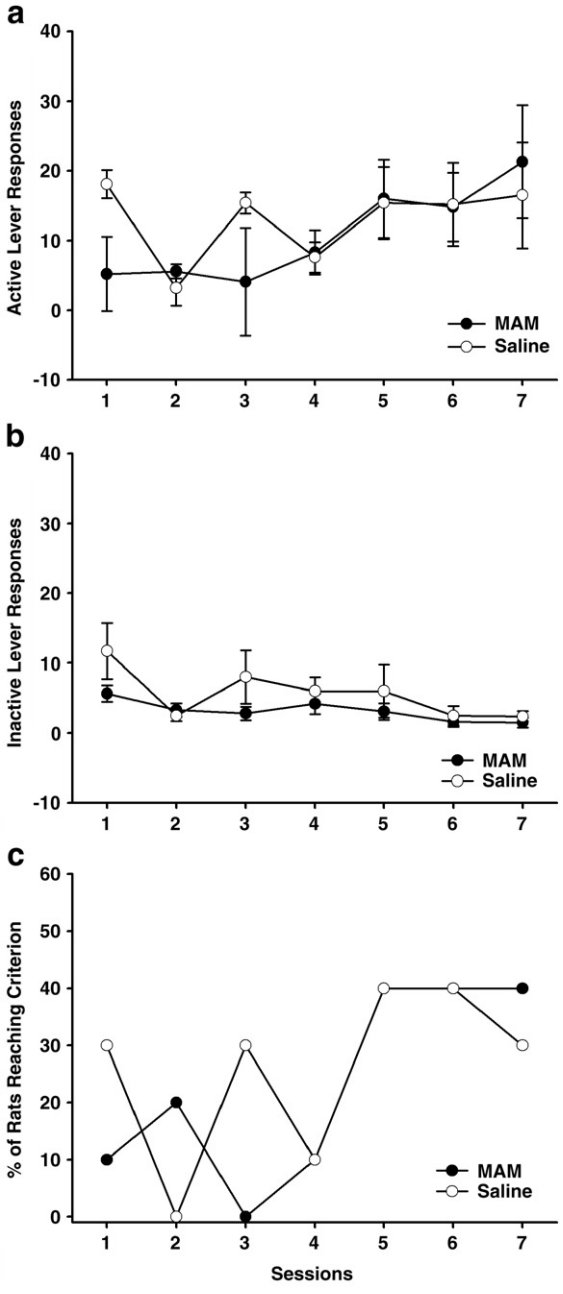

Fig. 1. Acquisition of cocaine self-administration in MAM versus saline treated animals during experiment 1. Top graph depicts response rate on the active lever, while the middle graph depicts response rate on the inactive lever. For the sessions of self-administration training depicted here, animals received a relatively low dose (0.1 mg/per infusion) of cocaine. No statistically significant differences were observed between the two groups. The bottom graph shows the percentage of animals reaching criterion (>15 responses) during acquisition of cocaine self-administration. No differences were observed between MAM and saline treated animals.

number of responses required for an infusion of cocaine increased with successive infusions. The progression was derived from the equation: ratio = $[5 \times e^{(0.2 \times \text{infusion no.})} - 5]$ yielding response ratios of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc. (Richardson and Roberts, 1996). Sessions lasted until either a period of 1 h without an infusion or 5 h had elapsed. The number of infusions earned before the breakpoint was measured. After 6 training sessions responding was stable for 0.25 mg/infusion, dose–response testing commenced. All rats received all doses of cocaine, and saline, in descending order for three sessions per dose (0.125, 0.0625, 0.03125 mg/infusion and saline). An additional session with the original 0.25 mg/infusion dose was given following the dose–response test in order to establish catheter patency. Twelve MAM treated and nine saline treated animals completed this phase of testing.

2.4.3.2. Brain weights. After PR training animals were overdosed with 0.5 ml of euthanol IV. Rats were immediately decapitated, their brains extracted and the parietal cortex, hippocampus, caudate nucleus and the nucleus accumbens were dissected and the weight of each region was recorded. The weight of these brain regions was measured to ensure the MAM treatment was successful because these regions have been shown to weigh significantly less in MAM treated rats (Featherstone et al., 2007).

2.4.4. Experiment 4

2.4.4.1. Cocaine-induced locomotor activity. Rats were habituated to the locomotor activity cages over three separate 120 min sessions. Drug testing used a within-subjects design, wherein each animal was given exposure to each dose of cocaine (saline, 5, 10 mg/kg and 20 mg/kg) on separate days. The pattern of drug exposure was counterbalanced with approximately equal numbers if animals were tested at each dose on each test day. Testing sessions commenced with a 30 min habituation period, after which time rats were injected with their respective dose of cocaine and activity was observed for a further 90 min. Drug sessions occurred every other day and during off days, animals were left undisturbed. As in experiment 3 the weights of each of the aforementioned brain regions were measured.

3. Results

3.1. Experiment 1

Fig. 1a and b shows the number of responses on the active and inactive levers during acquisition of cocaine self-administration in MAM and saline treated animals. Data were assessed using a 3 way repeated measures ANOVA, with treatment Group (MAM versus saline), Lever (active versus inactive) and Session as the independent variables. A significant main effect was found for Lever [$F(1,40) = 8.14$, $p<0.01$] and Session [$F(6,240) = 2.7$, $p<0.05$]. As well, a significant interaction was found for the Session by Group [$F(6,240) = 2.25$, $p<0.05$] and Session by Lever [$F(6,240) = 3.95$, $p<0.01$] interactions.

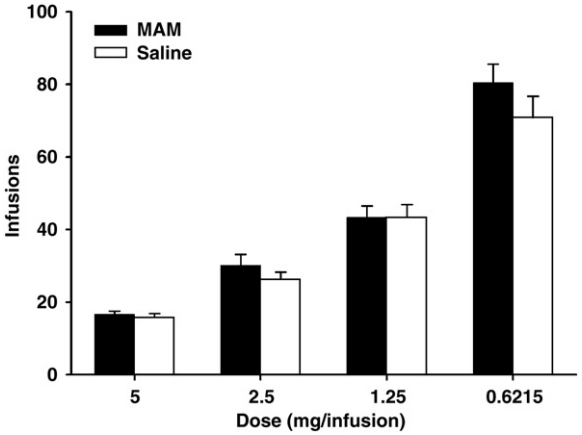

Fig. 2. Number of cocaine infusions in MAM versus saline treated animals during a dose–response test with varying doses of cocaine (0.0625, 0.1, 0.25, 0.5 mg/infusion) during experiment 2. While animals in both groups responded more frequently as the dose of cocaine increased, no differences were observed between the two groups.

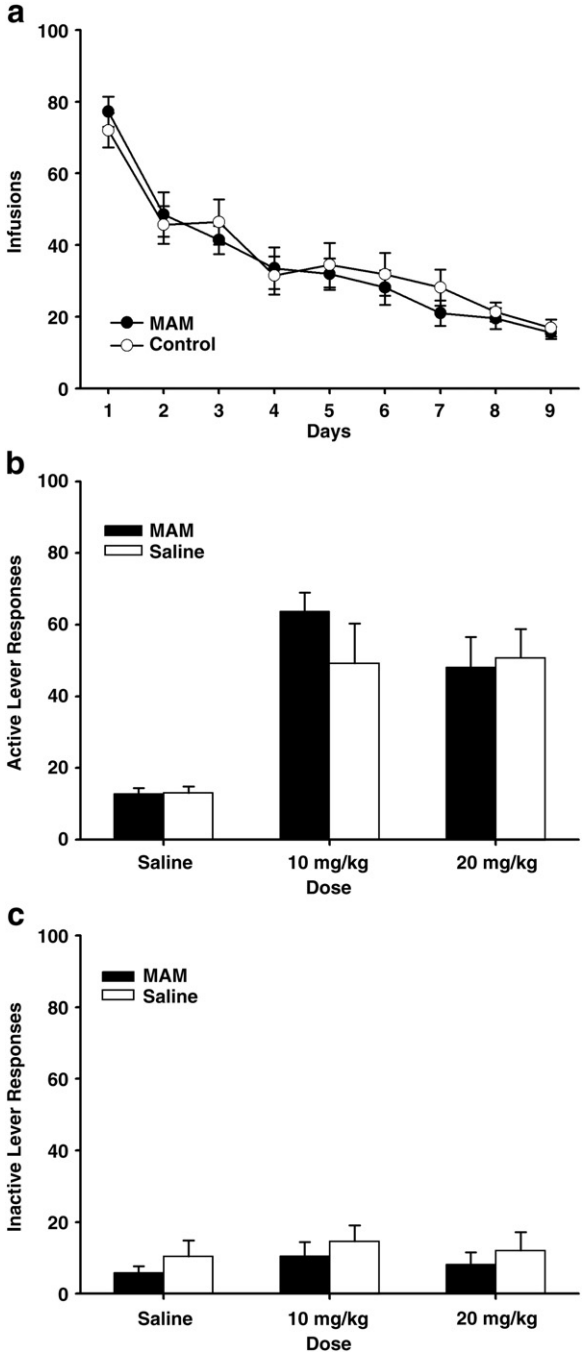

Fig. 3. Top graph shows the number of responses on the previously active lever during extinction training in MAM versus saline animals. No significant differences were observed between the two groups. The middle and bottom graphs depict cocaine-induced reinstatement of responding on the active and inactive levers in MAM and saline treated animals, respectively. On the day after extinction training, animals were given an injection of either saline, 10 mg/kg cocaine or 20 mg/kg of cocaine and responding was assessed. In comparison to responding following saline exposure, both groups showed significantly higher rates of responding on the active (but not inactive) lever following exposure to cocaine. No significant differences were observed between the two groups.

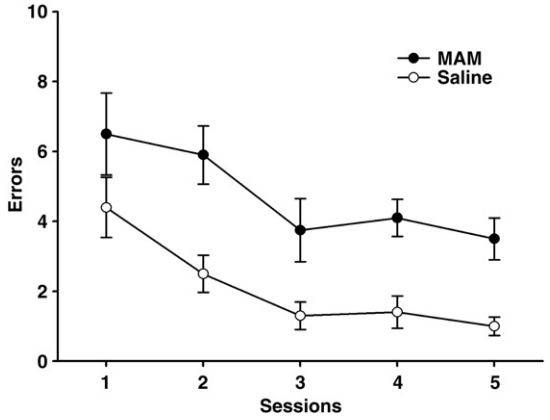

Fig. 4. Performance on the win-shift task in MAM and saline treated animals. MAM treated animals showed a significantly greater number of errors than saline treated animals ($p<0.05$).

Planned comparisons analyses found that saline treated rats had a higher response rate on the active lever on session one only [$F(1,40) = 6.07$, $p<0.05$]. No group differences were found for responses on the inactive lever. The percentage of animals that achieved greater than 15 infusions per session during the last three days of training were 36.4, 36.4, and 36.4 in MAM treated animals and 36.4, 36.4 and 27.3 in saline treated animals (Fig. 1b).

3.2. Experiment 2

Rats readily acquired cocaine self-administration with the 0.25 mg/infusion dose over 12 days. There was no group difference between MAM treated animals and controls. The average daily number of infusions over the last 3 days was 26.4 (SEM 1.52) for MAM treated animals and 25.9 (SEM 1.38) for saline treated animals.

3.2.1. Dose–response test

The effects of MAM treatment on cocaine self-administration across a range of infusion doses are shown in Fig. 2. A repeated measures ANOVA with Group and Dose as independent variables found a significant main effect of Dose [$F(3,81) = 170.16$, $p<0.001$], with infusion rate increasing as dose of cocaine decreased. No effect of Group, or Group by Dose interaction, shows that self-administration of cocaine was not altered by MAM at any infusion dose.

3.2.2. Extinction

Fig. 3a shows responses on the previously active lever during extinction of self-administration behaviour. A repeated measures ANOVA using Group and Session as independent variables found a significant main effect of Session [$F(8, 216) = 40.62$, $p<0.05$], with response rate decreasing over the course of training. Neither the main effect of Group nor the interaction term was significant, showing that the rate of extinction was not affected by MAM treatment.

3.2.3. Reinstatement

Fig. 3b and c illustrates the effects of priming injections of cocaine on responding in the previously active and inactive levers. Data were analyzed using a three way repeated measures ANOVA using Group, Dose and Lever (active versus inactive) as independent variables. A significant effect was found for Dose [$F(2,104) = 25.5$, $p<0.01$], and for Lever [$F(1,52) = 52.7$, $p<0.01$]. As well, a significant interaction was observed between Lever and Dose [$F(2,104) = 17.8$, $p<0.01$], but not for Group by Lever, Group by Dose or the three way interaction. Thus, while re-exposure to cocaine dose-dependently increased the rate of responding on the active lever, this increase was equivalent in MAM and saline treated animals.

3.2.4. Win-shift radial maze task

A repeated measures ANOVA was conducted on the error scores using Group and Session as independent variables. Data are shown in Fig. 4. A significant main effect was found for Group [$F(1,14) = 19.09$, $p<0.05$], with MAM treated animals showing higher rates of error than saline treated animals, and for Session [$F(4,56) = 8.50$, $p = 0.05$], with error rates decreasing as animals learned the task. The Group by Session interaction was not significant. Thus, gestational MAM treatment resulted in deficits in learning and/or performing the radial maze task.

3.3. Experiment 3

As in experiment 1, there was no group difference between MAM and saline rats on baseline responding on the FR1 schedule. All rats readily acquired self-administration of cocaine at the 0.25 mg/infusion dose on the PR schedule. Responding stabilized with an average of 15.56 ± 0.82 infusions for MAM rats and 16.370 ± 0.98 infusions for saline rats calculated over the last three baseline sessions. These infusion rates correspond to an average breaking point ratio of 158.47 ± 20.72 and 226.98 ± 71.78, respectively. There were no group differences or interactions across PR sessions, as demonstrated by a repeated measures ANOVA using group (MAM versus saline) and session as independent variables [$F(1,19) = 0.211$, p ns; $F(1,19) = 0.255$, p ns] (see Fig. 5a).

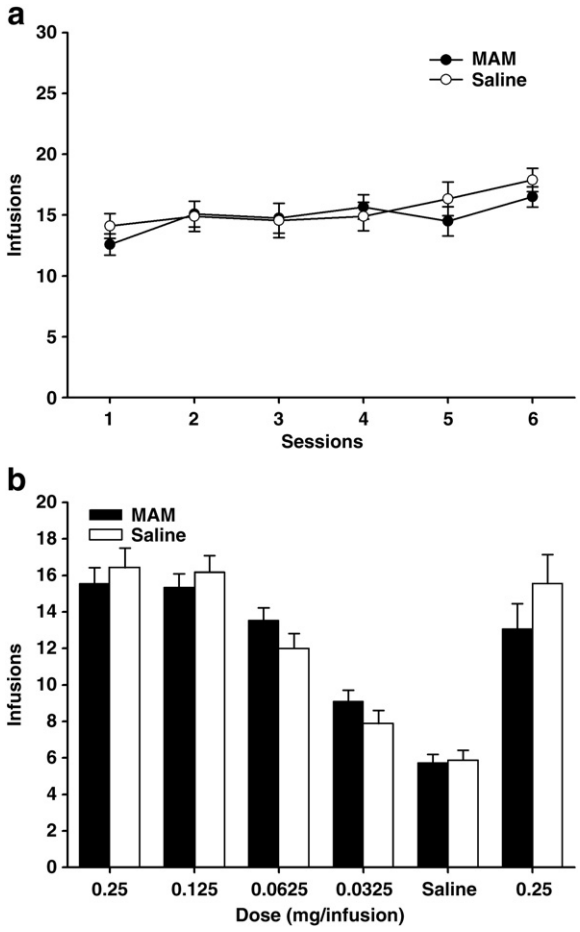

Fig. 5. Top graph depicts the number of infusions earned in MAM versus saline treated animals over 6 sessions of a progressive ratio schedule of reinforcement. No differences were observed between the two groups. Bottom graph shows the number of infusions earned in MAM versus saline treated animals during a dose–response test with varying doses of cocaine or saline (saline, 0.0325, 0.0625, 0.125, and 0.25 mg/infusion) following stabilization of responding on a progressive ratio schedule of reinforcement. While animals in both groups earned fewer infusions as the dose of cocaine decreased, no differences were observed between the two groups.

3.3.1. Dose–response test

The effects of MAM treatment on cocaine self-administration across a range of infusion doses are shown in Fig. 5b. A repeated measures ANOVA with Group and Dose as independent variables found a significant main effect of Dose [$F(4,76) = 105.87$, $p < 0.0001$], with the number of infusions decreasing as the dose of cocaine decreased. The main effect of Group, and the Group by Dose interaction were not significant showing that self-administration of cocaine was not altered by MAM at any infusion dose. Reinstating the 0.25 mg/infusion dose increased responding significantly from saline levels. A repeated measures ANOVA with Group and Dose as independent variables and infusions as the dependent measure showed a significant main effect of dose [$F(1,19) = 60.112$, $p = 0.0001$].

3.3.2. Brain weights

The effects of MAM on brain weight are shown in Fig. 7a. One-tailed *t*-tests were conducted for each brain region with group (MAM vs. saline) as the independent variable and weight (mg) as the dependent variable. MAM significantly reduced the weights of the parietal cortex [$t\ (19) = -3.14$, $p = 0.003$], hippocampus [$t\ (19) = -1.96$, $p = 0.03$] and the striatum [$t\ (19) = -1.92$, $p = 0.03$], but not the nucleus accumbens [$t\ (19) = 0.009$, $p =$ ns].

3.4. Experiment 4

3.4.1. Cocaine-induced locomotor activity

A repeated measures ANOVA with Session and Group as independent variables was conducted on the locomotor activity scores across the three habituation sessions. A significant main effect was observed for Session [$F(2,44) = 3.521$, $p = 0.038$], indicating that locomotor activity decreased across sessions. The amount of locomotor activity, and rate of habituation were not affected by MAM since the main effect of MAM, and the interaction between MAM and Session were not significant (data not shown). For cocaine-induced locomotor activity, a repeated measures ANOVA using Group and Dose as independent variables confirmed that cocaine increased locomotor activity [$F(3,66) = 26.825$, $p < 0.001$]. This response was not altered by MAM treatment; neither the main effect of Group, nor the Group × Dose interaction were significant. These data are depicted in Fig. 6.

3.4.2. Brain weights

The effects of MAM on brain weight are shown in Fig. 7b. One-tailed *t*-tests were conducted for each brain region with Group (MAM vs. saline) as the independent variable and weight (mg) as the dependent variable. MAM significantly reduced the weights of the parietal cortex [$t\ (22) = -3.33$, $p = 0.001$], hippocampus [$t\ (22) = -2.49$, $p = 0.01$] and the nucleus accumbens [$t\ (22) = -1.80$, $p = 0.04$], but not the striatum [$t\ (22) = -0.32$, $p =$ ns].

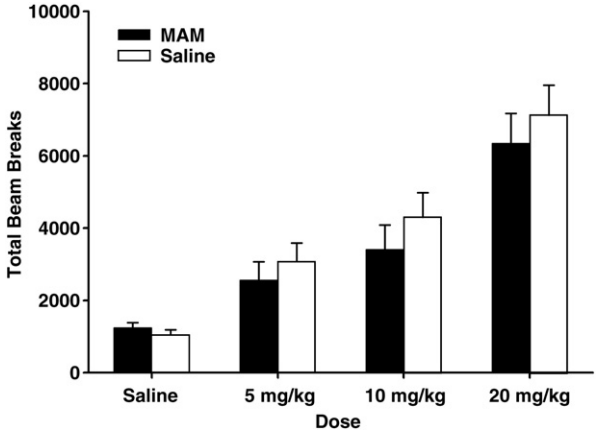

Fig. 6. Locomotor response to cocaine in MAM and saline treated animals. While the administration of 5 mg/kg, 10 mg/kg or 20 mg/kg cocaine significantly increased locomotor activity, this increase was equivalent between the two groups.

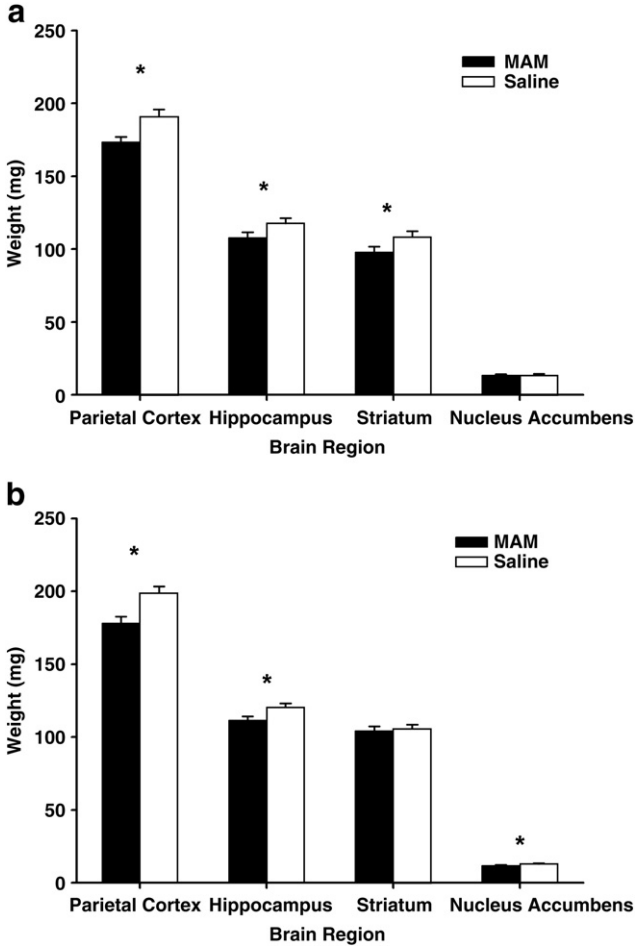

Fig. 7. The top graph shows brain weights (mg) in MAM and saline treated animals from experiment 3. MAM treated animals showed a significant reduction in brain weight in the parietal cortex, hippocampus and dorsal striatum (*$p < 0.05$), but not the nucleus accumbens. The bottom graph shows brain weights (mg) in MAM and saline treated animals from experiment 4. MAM treated animals showed a significant reduction in brain weight in the parietal cortex, hippocampus and nucleus accumbens ($p < 0.05$), but not the dorsal striatum.

4. Discussion

Much evidence suggests that individuals with schizophrenia show enhanced rates of substance abuse relative to unaffected individuals (Hambrecht and Hafner, 1996; Regier et al., 1990). This relationship is thought to stem from a shared neuropathology common to both substance abuse and schizophrenia (Chambers et al., 2001). The present study assessed whether gestational MAM exposure would enhance behavioural responses to cocaine in adulthood. Such enhancements, if present, would suggest that MAM treatment results in a heightened response to drugs of abuse, a change that is thought to underlie substance abuse disorders. In the present series of experiments, MAM treated animals failed to show a heightened behavioural response to cocaine in various self-administration paradigms and locomotor activity. However, gestational MAM treatment impaired hippocampal-dependent learning and memory on a win-shift radial maze task. Thus, under the conditions tested in the experiments MAM treatment did not reproduce the increased responsivity to drugs of abuse aspect of schizophrenia.

In experiment 1, MAM and saline treated animals showed a similar rate of acquisition of cocaine self-administration using a sub-threshold dose of cocaine. This was indicated both by similar rates of change in discrimination ratio over the course of training and number of animals reaching acquisition criterion. Previous studies have used this methodology, including the dose of cocaine, to successfully detect the effects of prior amphetamine or other stimulant exposure on subsequent sensitivity to the reinforcing properties of cocaine (Horger et al., 1990; Piazza et al., 1989; Pierre and Vezina, 1997). However, it could be argued that the procedure, and particularly the infusion dose, was too low to detect any changes in sensitivity to the reinforcing properties of cocaine present in MAM treated animals. In order to address this question further we examined whether MAM treatment altered established self-administration of cocaine under a FR1 schedule with different unit doses of cocaine. Again, MAM and saline treated animals showed similar levels of responding across the various doses of cocaine.

Responding under an FR1 schedule of reinforcement has been criticized as being an ambiguous measure of changes in reinforcer efficacy, since either an increase or decrease in response rate could be interpreted as reflecting an increase in reinforcer efficacy (Arnold and Roberts, 1997). Additionally, it has been shown that response rate on an FR schedule is not always sensitive enough to detect changes in reinforcer efficacy. For example, Suto et al. (2002) found no change in response rate on a fixed ratio schedule following direct infusions of amphetamine into the VTA. In contrast, when tested on a PR schedule amphetamine exposed animals showed an increase in breakpoint for cocaine reinforcement, suggesting that prior amphetamine exposure produced an increase in the reinforcing properties of cocaine. However there were no differences between MAM and saline treated animals responding for cocaine available on a PR schedule. Both groups showed near identical breakpoints, and these appeared to be stable across several sessions of training. This failure to detect an increase in breakpoint in MAM treated animals strongly suggests that the reinforcing efficacy of cocaine was similar between MAM and saline treated animals. When considered alongside the lack of effect of MAM treatment on FR1 schedule responding and cocaine-induced locomotor activity these data suggest that MAM treatment does not produce an increase in sensitivity to the rewarding properties of cocaine. Thus, it would appear that MAM may not be a suitable model of the increased rates of substance abuse that are seen in schizophrenia, especially in relationship to cocaine.

Following the dose–response test in experiment 2 animals were given several sessions of extinction training during which responding on the active lever no longer delivered an infusion of cocaine. MAM treated animals showed a similar rate of extinction to saline treated animals, suggesting that MAM treatment did not alter this behaviour. While the failure to find a difference in extinction rate between the two groups is consistent with the apparent lack of effect of MAM treatment on acquisition of cocaine self-administration seen earlier, some deficit in this behaviour could be expected given the ability of MAM to induce changes in the PFC (Flagstad et al., 2004; Moore et al., 2006) and the demonstrated role of this area of the brain in extinction (Morgan et al., 1993; Dalley et al., 2004). Additionally, deficits in extinguishing drug-seeking behaviour have been reported in adult animals given neonatal lesions of the ventral hippocampus (Chambers and Self, 2002), a manipulation that in some ways appears to induce neural changes similar to those induced by MAM treatment. It is not clear why extinction was not altered in MAM treated animals in the present experiment, although it is possible that animals did not receive enough training on the task or received too little exposure to cocaine for differences in extinction rates to be seen. It is also possible that such differences would have emerged if training had been continued over a greater number of sessions.

No differences were observed between MAM and saline treated animals on cocaine-induced reinstatement of responding. Animal studies of reinstatement of drug seeking are thought to model the process of relapse in humans (Stewart, 2003). In human studies, cocaine addicted individuals with schizophrenia show increased rates of cocaine craving in response to the presentation of drug associated cues, relative to addicts without schizophrenia (Smelson et al., 2002). Furthermore, increases in drug craving are thought to increase the probability of relapse to drug seeking (Stewart, 2003), suggesting that rates of relapse should be higher in people with schizophrenia. Given that MAM treated animals showed similar rates of drug-induced reinstatement of responding for cocaine as were shown in saline animals it would appear to be the case that MAM does not change this aspect of substance abuse and further strengthens the argument that there was no change in sensitivity to the rewarding effects of cocaine following MAM treatment.

Finally, it is possible that the doses of cocaine used in the reinstatement study were too high for detecting differential sensitivity to the priming effect of cocaine on reinstatement. This notion is supported by the fact that animals showed similar levels of reinstatement at both the 10 and 20 mg/kg doses, suggesting that response rate had peaked at the 10 mg/kg dose. However, the finding of similar levels of reinstatement across the doses used in the current study was unexpected given that previous work in our lab and others has shown that the 20 mg/kg dose generally produces higher levels of reinstatement than the 10 mg/kg dose (Fletcher et al., 2002; Placenza et al., 2005). While it remains possible that some differences in reinstatement would have emerged with a lower dose of cocaine, this explanation can not account for the lack of differences observed in the remaining experiments. Experiment 1 used an infusion dose of cocaine that barely supported self-administration in control rats. Experiment 2 used a range of cocaine doses and responding in both groups was clearly dose dependent. Similarly for experiment 3 a dose–response curve was generated for rats responding on a PR schedule. Again, a clear dose–response relationship was observed in both groups, with a wide range in breakpoints. Much the same pattern was observed in experiment 4, which showed a clear dose–response relationship for the ability of cocaine to stimulate locomotor activity. Given the range of doses used in all of these studies, it seems highly improbable that any of the behavioural measures were limited by floor or ceiling effects. Thus, the similarity of response to cocaine-induced reinstatement in MAM treated and control animals is consistent with the lack of differential sensitivity to cocaine seen in tests of cocaine self-administration and cocaine-induced locomotor activity, where clear dose–response relationships were observed.

Several lines of evidence suggest that the null effect of MAM treatment on the effects of cocaine reported presently was not due simply to a general lack of effect of MAM treatment. First, in experiments 3 and 4 brain weights were measured and these showed significant differences in brain areas previously reported to be disrupted in MAM treated animals, such as the hippocampus and parietal cortex (Featherstone et al., 2007). While brain weights were not measured for the animals in experiments 1 and 2, brain weight measurements have been previously reported from siblings of the animals used in experiment 1 (Featherstone et al., 2007), and these showed decreases in hippocampal and cortical tissue size that were generally consistent with those reported in other laboratories (Flagstad et al., 2004). As well, significant impairments were observed on an attentional set-shifting task known to be sensitive to prefrontal (Birrell and Brown, 2000) and parietal (Fox et al., 2003) cortex damage in these same animals (Featherstone et al., 2007). Second, MAM treated animals showed a strong deficit on the hippocampal-dependent win-shift task in the present study, a finding that is consistent with the reported effects of MAM on hippocampal development (Moore et al., 2006).

In contrast to the current study, two previous studies have reported evidence of enhanced nucleus accumbens dopamine function in E17 treated MAM animals, as indicated by a heightened locomotor response to an amphetamine challenge (Flagstad et al., 2004; Moore et al., 2006), heightened extracellular dopamine release in response to amphetamine (Flagstad et al., 2004) and changes in dopamine dependent

behaviours such as prepulse inhibition of the acoustic startle response (Le Pen et al., 2006). A number of explanations could be put forth to explain these differences. First, it is possible that exposure to different environmental factors, such as stress or rearing conditions, could influence the magnitude of behavioural deficits induced by MAM treatment, and it is possible that the presence of these variables differed between laboratories. Second, all of the studies showing enhanced responsiveness to dopamine agonist drugs in MAM treated animals have used amphetamine rather than cocaine. A great deal of evidence suggests that the reinforcing and stimulant effects of amphetamine and cocaine are mediated by a common neurochemical substrate. Both drugs elevate extracellular dopamine (Di Ciano et al., 1995; Kuczenski and Segal, 1989), and the effects of both drugs are altered by dopamine-depleting lesions of the nucleus accumbens (Lyness et al., 1979; Pettit et al., 1984; Roberts et al., 1980) as well as by dopamine receptor antagonists (Caine and Koob, 1994; Corrigall and Coen, 1991). However, there are some differences in the neurochemical and behavioural effects induced by these drugs that might make amphetamine a more sensitive tool for detecting the types of neural changes, including apparent increased functional sensitivity of dopamine systems, which are altered by MAM treatment. Pharmacologically, cocaine acts primarily to block the dopamine transporter, whereas the main action of amphetamine is to release dopamine. Cocaine also elevates brain serotonin levels by blocking the serotonin transporter (Müller et al., 2003), whereas effects of amphetamine are typically seen only at higher doses that induce stereotypy (Kuczenski and Segal, 1989). Behaviourally, infusions of amphetamine into the nucleus accumbens are sufficient to support self-administration (Hoebel et al., 1983) and conditioned place preference (Carr and White, 1986), while infusions of cocaine into the nucleus accumbens are unable to support either behaviour (Goeders et al., 1986; Hemby et al., 1992a). Infusion of cocaine into the PFC can support self-administration (Goeders et al., 1986; Goeders and Smith, 1983) and conditioned place preference learning (Hemby et al., 1992b), and lesions of the PFC differentially affect the development of sensitization to the stimulant effects of cocaine and amphetamine (Tzschentke and Schmidt, 2000). Overall, the results suggest that while MAM treatment produces some effects that indicate increased functional activity of the mesocorticolimbic dopamine system (Flagstad et al., 2004; Moore et al., 2006) these effects appear to be insufficient to alter the reinforcing or psychomotor stimulant effects of cocaine.

In summary, the present study assessed the effects of gestational MAM treatment on several tasks known to be sensitive to changes in sensitivity to the rewarding effects of cocaine thought to underlie abuse of this drug. At no time did MAM treated animals show any difference in performance of any of these tasks relative to saline treated animals, suggesting that, at least under the present conditions, MAM treatment did not reproduce any behavioural changes analogous to the increased rates of drug use in schizophrenia. At present, the reasons for this null result are unclear. We are currently examining a number of possible factors that could account for the apparent discrepancy between results reported here and those previously reported by other labs.

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