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(+)-Methamphetamine-induced monoamine reductions and impaired egocentric learning in adrenalectomized rats is independent of hyperthermia

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Abstract

Methamphetamine (MA) is widely abused and implicated in residual cognitive deficits. In rats, increases in plasma corticosterone and egocentric learning deficits are observed after a one-day binge regimen of MA (10 mg/kg $\times$ 4 at 2 h intervals). The purpose of this experiment was to determine if adrenal inactivation during and following MA exposure would attenuate the egocentric learning deficits in the Cincinnati water maze (CWM). In the first experiment, the effects of adrenalectomy (ADX) or sham surgery (SHAM) on MA-induced neurotoxicity at 72 h was determined. SHAM-MA animals showed typical patterns of hyperthermia whereas ADX-MA animals were normothermic. SHAM-MA and ADX-MA treated animals both showed increased neostriatal glial fibrillary acidic protein and decreased monoamines in the neostriatum, hippocampus, and entorhinal cortex. In the second experiment, SHAM-MA and ADX-MA treated groups showed equivalently impaired CWM impairments two weeks post-treatment (increased latencies, errors, and start returns) compared to SHAM-saline (SAL) and ADX-SAL groups with no effects on novel object recognition, elevated zero maze, or acoustic startle/prepulse inhibition. Post-testing, monoamine levels remained decreased in both MA-treated groups in all three brain regions, but were not as large as those observed at 72 h post-treatment. The data demonstrate that MA-induced learning deficits can be dissociated from drug-induced increases in plasma corticosterone or hyperthermia, but co-occur with dopamine and serotonin reductions.

Keywords

Methamphetamine; dopamine; serotonin; egocentric learning; maze learning; swimming maze; Cincinnati water maze; Zero maze; acoustic startle; corticosterone; glial fibrillary acidic protein; GFAP

Introduction

Methamphetamine (MA) is an addictive stimulant that has become increasingly popular (EMCDDA, 2007; Johnston et al., 2008; Johnston et al., 2009). In recent years, however it

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has become appreciated that prolonged use results in long-lasting neurochemical and cognitive alterations (Baicy and London, 2007; Barr et al., 2006; Chang et al., 2007; Meredith et al., 2005). Cognitively, MA abusers may exhibit impairments of working memory, attention, and executive functioning even after prolonged abstinence (Baicy and London, 2007; Barr et al., 2006; Ellinwood, 1967; Meredith et al., 2005; Yoshida, 1997). The underlying mechanism(s) for these changes are not understood, although autopsy and neuroimaging studies reveal reductions of brain monoamines and associated reuptake transporters after chronic MA use (Baicy and London, 2007; Barr et al., 2006; Chang et al., 2007; Meredith et al., 2005). In addition, MA induces cortisol release (Fehm et al., 1984). Increased corticosterone release has well-established effects on cognitive function (Lupien and McEwen, 1997; Raber, 1998). Repeated stress or exogenous corticosterone exposure induces structural changes in the hippocampus and impaired spatial learning and memory in the Morris water maze (MWM (Sousa et al., 2000)). Partial lesions of the hippocampus, that otherwise lead to impaired MWM memory, can be prevented by treatment with metyrapone which inhibits corticosterone synthesis (Roozendaal et al., 2001). There are many examples of how stress and elevated corticosterone release adversely affect behavior (see (Carrasco and Van de Kar, 2003; McEwen, 2000; Sapolsky, 1996; Sapolsky and Meaney, 1986). However, the role of corticosterone release in MA-induced cognitive deficits is unknown and determining its role is the purpose of the present experiment.

As one model of the cognitive deficits, we recently showed that adult male rats receiving a binge regimen of MA (10 mg/kg $\times$ 4 every 2 h) exhibit deficits in egocentric learning in the Cincinnati water maze (CWM), i.e., in the absence of distal cues (Herring et al., 2008). Others have reported binge MA-induced impairments in novel object recognition (Belcher et al., 2005; Bisagno et al., 2002; He et al., 2006; Schroder et al., 2003), however we and others have reported complete or partial sparing of spatial learning in the MWM (Friedman et al., 1998; Herring et al., 2008; Schroder et al., 2003). Experiments on another substituted amphetamine, fenfluramine, show CWM deficits as well that can be blocked by surgical or chemical adrenalectomy (ADX) (Skelton et al., 2004; Williams et al., 2002) however, these results may be different because in these cases distal cues were present during testing in the CWM. As with fenfluramine, MA treatment increases corticosterone release and reduces serotonin (5-HT) (Herring et al., 2008). On the other hand, MA differs from fenfluramine inasmuch as MA causes reductions in dopamine and increases in both glial fibrillary acidic protein (GFAP) and core body temperature, whereas fenfluramine does not. The present experiment was designed to determine whether preventing MA-induced corticosterone release by prior ADX alters the effects of the drug on egocentric learning (in the absence of distal cues), monoamines, hyperthermia, and GFAP compared to sham-operated (SHAM) MA-treated rats and controls.

Inhibitors of corticosterone release were not employed because they can cause hypothermia (Healy et al., 1999; Skelton et al., 2004) thereby interfering with the effects of MA. Hyperthermia is correlated with MA-induced neurotoxicity and inhibited or attenuated reductions in striatal dopamine are observed in animals treated in low ambient temperature conditions (Ali et al., 1994; Bowyer et al., 1992; Bowyer et al., 1994). ADX does not produce hypothermia alone or in combination with MA treatment (Makisumi et al., 1998). We also assayed GFAP as a marker for astrogliosis to ensure that our dosing regimen was inducing neurotoxicity (O'Callaghan and Miller, 2002).

Methods

Animals

Two experiments were conducted in which treatment and housing conditions were identical. Subjects were male Sprague-Dawley CD IGS rats (325 – 350 g, Charles River Laboratories,

Raleigh, NC) that were purchased from the supplier after ADX or SHAM surgery. Animals were acclimated to the vivarium for 1–2 weeks prior to drug treatment (temperature, $19 \pm 1^\circ\text{C}$, $50 \pm 10\%$ humidity, and 14 h light: 10 h dark cycle (lights on at 600 h)), housed in pairs in cages measuring $46 \times 24 \times 20$ cm, separated 3–7 days before drug treatment, and maintained in this manner for the remainder of each experiment (Herring et al., 2008). Food and water were freely available except during treatment. All procedures were conducted in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee. The vivarium is accredited by AAALAC.

Methamphetamine Administration

(+)-Methamphetamine-HCl (10 mg/kg freebase, obtained from NIDA and > 95% pure) or isotonic saline (SAL) was administered in 4 doses with a 2 h inter-dose interval beginning at 900 h. Each animal was weighed immediately prior to the first injection (Herring et al., 2008). The mean ambient room temperature during MA treatment for Experiment-1 was $22.6 \pm 1^\circ\text{C}$ and for Experiment-2 was $22.8 \pm 1^\circ\text{C}$.

Body Temperature Monitoring

Body temperatures of animals were measured with temperature transponders (IPTT-300: Bio Medic Data Systems, Seaford, DE) that were implanted under light isoflurane anesthesia subcutaneously in the dorsum 3 days prior to treatment. Transponders were used to prevent the stress of rectal temperature measurements (Bae et al., 2007; Balcombe et al., 2004). Animals were cooled in a shallow water bath if temperature readings reached 40.2°C as described previously (Herring et al., 2008). Two hours after the last dose, animals were returned to the colony room.

Experiment-1

The effects of MA in ADX or SHAM animals were compared 3 days following treatment (Bowyer et al., 1992; Marshall et al., 2007). The experimental treatment groups were as follows: ADX-SAL, $n = 10$; ADX-MA, $n = 12$, SHAM-SAL, $n = 10$, SHAM-MA, $n = 10$. A few animals died resulting in final group sizes of: ADX-SAL, $n = 9$, ADX-MA, $n = 7$, SHAM-SAL, $n = 10$, and SHAM-MA, $n = 6$. Seventy-two hours after the last dose, animals were transported individually to an adjacent suite in < 30 s, decapitated (Holson, 1992), and blood collected in polyethylene tubes containing 2% EDTA (0.05 ml/tube). The brain was removed, placed over ice, and neostriatum (caudate and putamen) and hippocampus were dissected as described previously (Williams et al., 2007). The entorhinal cortex was dissected by making a coronal cut at the posterior extent of the mammillary body and another cut 2 mm further posterior. From this 2 mm section, the entorhinal cortex was removed bilaterally by making a cut at the rhinal fissure and removing the cortical tissue inferior to this cut to the tip of the corpus callosum. All tissues were collected between 900 and 1100 h. Monoamines in brain tissue were assayed by HPLC, GFAP in neostriatum was assayed by Western blot, and corticosterone was assayed by EIA as described previously (Herring et al., 2008).

Experiment-2

There were four treatment groups as above: SHAM-SAL ($n=16$), SHAM-MA ($n=17$), ADX-SAL ($n=13$), and ADX-MA ($n=13$). Animals were evaluated behaviorally at the following intervals after the last dose: stereotypy (0–30 h), elevated zero maze (3 days), novel object recognition (7–10 days), straight channel swimming (13 days), Cincinnati water maze (CWM; 14–31 days), and prepulse inhibition of the acoustic startle response (32–33 days) (Herring et al., 2008). All tests were performed during the light phase of the light/dark cycle, and apparatus were cleaned with 70% ethanol between subjects.

Stereotypy Monitoring

Stereotypy was assessed in order to document whether ADX altered the acute effects of MA. Animals were scored for stereotypy immediately after the last dose in 30 min intervals for 2 h, and then again at 10, 12, 24 and 30 h. The following scale was used: 0 = sleeping, 1 = resting, eyes open, not moving, 2 = active (grooming, exploring), 3 = stereotypy. Stereotypy was defined as oral (chewing, licking, or biting), sniffing (focused), and/or repetitive head and paw movements. At each of the designated times, the experimenter entered the room, observed the status of the animal, and recorded an appropriate score at that moment.

Elevated Zero Maze

Because MA-treated animals have increased corticosterone 3 days after MA treatment (Herring et al., 2008), we examined anxiety levels at 3 days using the elevated zero maze (Williams et al., 2003). Animals were placed in the middle of one of the closed quadrants and allowed 5 min to explore. Overhead fluorescent lighting was turned off during testing and the room was illuminated by a single halogen lamp. Each trial was digitally recorded and later scored using ODLog software (Macropod software) for time spent in the open quadrants.

Novel Object Recognition

The novel object recognition (NOR) procedure began 7 days after MA treatment (Herring et al., 2008). Animals were habituated to the testing arena for 3 days and tested for novel object recognition on the fourth day using the previously described procedure (Clark et al., 2000) in which animals received exposure to two identical objects for up to 10 min or until they accumulated 30 s of object exploration (familiarization). One hour later object preference was assessed using one familiar and one novel object for up to 10 min or until 30 s of object exploration occurred. The dependent variable was time spent attending to the objects.

Cincinnati Water Maze

Prior to assessing animals in the CWM, on day 13 after MA treatment, animals were tested for swimming ability in a 244 cm straight swimming channel for 4 trials with a maximum time limit of 2 min (Vorhees et al., 2008). CWM performance was tested for 18 days as previously described (Vorhees et al., 2008). Briefly, animals have to find their way through a 9-unit multiple T maze to locate an escape ramp. Animals are tested under complete darkness in order to obscure distal cues such that animals must rely on egocentric cues to find the escape. Two trials/day were given with a maximum time of 5 min/trial with at least a 5 min intertrial interval when animals failed to locate the escape within the time limit. Latency to escape, number of errors, and number of start returns were recorded. An error was defined as a head and shoulder entry in a dead-end arm of one of the Ts of the maze. The poorest performing animal among all groups committed 58 errors within the 5 min trial. A few animals stopped searching in the last min or two and remained within one arm of a T. For these animals an error score of 58 + 1 was assigned to correct for failure-to-search behaviors.

Acoustic Startle Response/Prepulse Inhibition (ASR/PPI)

ASR was measured in an SR-Lab apparatus (San Diego Instruments, San Diego, CA). Rats were placed in an acrylic cylindrical chamber mounted on a platform with a piezoelectric force transducer attached to the underside. The platform was located inside a sound-attenuated chamber with background white noise of 70 dB. A 5 min acclimation period preceded test trials. Each animal received a 4 × 4 Latin square sequence of trials that were of 4 types: no stimulus, startle stimulus, 74 dB prepulse + startle stimulus, or 76 dB prepulse +

startle stimulus. Each set of 16 trials was repeated 3 times for a total of 48 trials. Trials of the same type were averaged. The inter-trial interval was 8 s. The startle signal was a 20 ms 110 dB SPL mixed frequency burst or hiss. The startle recording window was 100 ms from startle signal onset. Prepulses preceded the startle stimulus by 70 ms from prepulse onset to startle signal onset.

Tissue collection

Five days following the completion of behavioral testing, animals were brought to an isolated suite, decapitated, and neostriatum, hippocampus, and entorhinal cortex collected as described above and assayed for monoamines.

Statistics

The CWM, straight channel, stereotypy, and temperature data were analyzed using factorial analysis of variance (ANOVA), general linear model or mixed linear model (GLM or Mixed, SAS Institute, Cary, NC). Treatment (MA or SAL) and Surgery (ADX or SHAM) were between-subject factors and day (CWM), trial (straight channel), or time (temperature or stereotypy) were within-subject factors. Novel object recognition, corticosterone, and neurotransmitter data were similarly analyzed but had no repeated measure factor. GFAP data were expressed against the appropriate control, either SHAM or ADX, and compared by t-test. The Greenhouse-Geisser correction was used in instances in which repeated measure ANOVA variance-covariance matrices were significantly non-spherical. Significance was set at $p \leq 0.05$ and trends were noted if $p < 0.10$. Data are presented as group means $\pm$ SEM. Only those interactions that include Treatment are presented.

Results

Body Temperatures (Experiment-1 and 2)

In Experiment-1, there were significant effects of Treatment, $F(1,27) = 9.88$, $p < 0.001$, Surgery, $F(1,27) = 19.65$, $p < 0.001$, Time ($p < 0.001$), and Treatment $\times$ Surgery $\times$ Time, $F(17,459) = 4.06$, $p < 0.01$. Examination of the interaction revealed that SHAM-MA animals were hyperthermic relative to SHAM-SAL animals from 30–510 min after the first dose ($p < 0.05$; Fig. 1A). In contrast, ADX-MA animals demonstrated a short-term reduction in body temperature compared to ADX-SAL animals at 30–90 min and a slight increase at 510 min after the first dose ($p < 0.05$; Fig. 1B).

In Experiment 2, a similar pattern of temperature changes was observed. There were significant effects of Treatment, $F(1,34) = 9.77$, $p < 0.01$, Time ($p < 0.0001$), Surgery, $F(1,34) = 7.49$, $p < 0.01$, and Treatment $\times$ Surgery $\times$ Time, $F(17,578) = 2.99$, $p < 0.01$. SHAM-MA animals displayed hyperthermia compared to SHAM-SAL animals from 30–510 min after the first injection ($p < 0.05$; Fig. 1C). There were no significant body temperature changes in ADX-MA animals compared to ADX-SAL animals (Fig. 1D).

Experiment 1

Corticosterone—Corticosterone was significantly increased in SHAM-MA animals compared to SHAM-SAL animals 3 days after dosing, Treatment, $F(1,29) = 7.08$, $p < 0.01$, and Treatment $\times$ Surgery, $F(1,29) = 6.57$, $p < 0.05$. ADX animals, by contrast, showed significantly decreased levels of corticosterone (at the limit of EIA detection) compared to SHAM animals, Surgery, $F(1,29) = 18.39$, $p < 0.001$. Mean $\pm$ SEM (ng/ml) plasma concentrations: SHAM-SAL = 16.0 ± 2.9 ; SHAM-MA = 56.1 ± 20.6 ; ADX-SAL = 2.8 ± 0.3 ; ADX-MA = 3.5 ± 0.7 .

Monoamines—In the neostriatum MA-treated (SHAM-MA and ADX-MA combined) groups had 60% decreased dopamine (DA), 52% decreased dihydroxyphenylacetic acid (DOPAC), 57% decreased 5-HT, and 54% decreased 5-hydroxyindolacetic acid (5-HIAA) compared to SAL-treated (SHAM-SAL and ADX-SAL combined) groups (Figure 2A–D, respectively) (Treatment $F(1,32)$: DA = 85.1; DOPAC = 39.6; 5-HIAA = 30.4, and $F(1,30)$: 5-HT = 34.2; $p < 0.0001$). The main effect of Surgery was not significant for DA, DOPAC, or 5-HIAA but was for 5-HT, $F(1,32) = 4.4$, $p < 0.05$; the combined ADX groups had higher 5-HT levels than the combined SHAM groups. None of the interactions of Treatment $\times$ Surgery were significant.

In the hippocampus, MA treatment resulted in 62% decreased 5-HT (Fig. 2E), Treatment, $F(1,32) = 55.8$, $p < 0.0001$, and 73% decreased 5-HIAA (Fig. 2F), Treatment, $F(1,32) = 45.4$, $p < 0.0001$, compared to SHAM-SAL and ADX-SAL groups, 3 days following treatment. Further, the combined ADX groups had higher hippocampal 5-HT and 5-HIAA compared to the combined SHAM groups, main effect of Surgery, $F(1,32) = 4.2$ and 6.4 , respectively, $p < 0.05$. There were no significant interactions. Direct comparison between the SHAM-MA and ADX-MA treated groups showed that the ADX-MA groups had slightly smaller reductions in 5-HIAA than the SHAM-MA group, $t(18) = 2.3$, $p < 0.05$, with no differences between the two groups for 5-HT.

In the entorhinal cortex, there was a main effect of Treatment such that the combined SHAM-MA and ADX-MA groups showed 71% 5-HT decreases (Fig. 2G), Treatment, $F(1,22) = 16.2$, $p < 0.001$, and 53% 5-HIAA decreases (Fig. 2H), Treatment, $F(1,22) = 20.8$, $p < 0.001$, 3 days following treatment compared to the combined SAL groups. No significant main effect of Surgery or interaction of Treatment $\times$ Surgery was observed. Direct comparison of the SHAM-MA and ADX-MA groups showed no significant difference for 5-HT or 5-HIAA. There were no significant effects on DA levels. The means $\pm$ SEM (pg/mg tissue) for DA are: SHAM-SAL, 84 ± 23 ; SHAM-MA, 120 ± 36 ; ADX-SAL, 103 ± 17 ; ADX-MA, 85 ± 26 .

Glial Fibrillary Acidic Protein—GFAP was assayed in the neostriatum and expressed as percent control (Fig. 3). The SHAM-MA and ADX-MA groups did not differ from one another, however, the combined SHAM-MA + ADX-MA groups differed significantly from the combined SHAM-SAL + ADX-SAL groups, $t(27) = 4.36$, $p < 0.001$.

Experiment-2

Stereotypy—For stereotypy Treatment, $F(1,58) = 813.5$, $p < 0.0001$, and Time, $F(8,464) = 33.6$, $p < 0.0001$, were significant main effects, whereas Surgery was not. Multiple interactions were also significant. These were the Treatment $\times$ Surgery, $F(1,58) = 7.5$, $p < 0.01$, Treatment $\times$ Time, $F(8,464) = 25.4$, $p < 0.0001$, and Surgery $\times$ Time, $F(8,464) = 6.5$, $p < 0.0001$, while the Treatment $\times$ Surgery $\times$ Time showed a non-significant trend, $F(8,464) = 1.8$, $p < 0.07$. The Treatment $\times$ Surgery interaction was followed up with slice ANOVAs for each surgical condition, one comparing the MA groups and one comparing the SAL groups. Comparison of the SHAM-MA to ADX-MA group was significant, $F(1,58) = 9.1$, $p < 0.01$, whereas the comparison of the SHAM-SAL to ADX-SAL was not. Since time did not interact, the data are shown in Fig. 4 averaged across time. As can be seen, the ADX-MA group showed slightly higher scores on the stereotypy scale than did the SHAM-MA group.

Elevated Zero Maze—No significant effects of Treatment, Surgery, or interactions among these factors were observed for time spent in open (SHAM-SAL = 73.9 ± 10.0 s, SHAM-MA = 57.0 ± 10.1 s, ADX-SAL = 58.1 ± 12.1 s, and ADX-MA = 68.7 ± 14.2 s), or

any other measure of performance (latency to first open entry, number of transitions, or head dips).

Novel Object Recognition—No differences were observed between groups in time spent with either object during familiarization. During the test phase, no significant effects of Treatment, Surgery, or interactions of these factors were obtained for time attending to the novel object (s): SHAM-SAL = 20.1 ± 1.4 ; SHAM-MA = 19.9 ± 0.9 ; ADX-SAL = 21.0 ± 1.5 ; ADX-MA = 23.2 ± 1.3 .

Straight Channel Swimming—All animals swam to the escape ladder progressively faster across successive trials (main effect of trials, $p < 0.0001$). There were no significant effects of Treatment, Surgery, or interactions among these factors. Mean $\pm$ SEM swimming times (s) averaged across trials for each group were: SHAM-SAL = 17.1 ± 1.9 ; SHAM-MA = 20.4 ± 1.8 ; ADX-SAL = 20.1 ± 1.9 ; ADX-MA = 21.3 ± 1.9 .

Cincinnati Water Maze—For escape latency in the CWM, there were significant effects of Treatment, $F(1,53) = 36.4$, $p < 0.0001$, Day, $F(17,901) = 145.8$, $p < 0.0001$, and Treatment $\times$ Day, $F(17,901) = 12.9$, $p < 0.0001$, but no main effect of Surgery and no interactions of Treatment $\times$ Surgery, Surgery $\times$ Day, or Treatment $\times$ Surgery $\times$ Day. As shown in Fig. 5A–B, both MA-treated groups (SHAM-MA and ADX-MA, respectively), regardless of Surgery, had longer latencies than both SAL-treated groups (SHAM-SAL and ADX-SAL) beginning on day 6 and continuing throughout the remainder of the test ($p < 0.01$). Analysis of errors was similar, that is, significant effects were seen for Treatment, $F(1,53) = 30.9$, $p < 0.0001$, Day, $F(17,901) = 118.3$, $p < 0.0001$, and Treatment $\times$ Day, $F(17,901) = 9.2$, $p < 0.0001$, but no main effect of Surgery or interactions between Surgery $\times$ Day, or Treatment $\times$ Surgery $\times$ Day were present. Beginning on day 5 and thereafter, both MA-treated groups (SHAM-MA and ADX-MA), regardless of Surgery, committed more errors than both SAL-treated groups (SHAM-SAL and ADX-SAL) ($p < 0.01$; Fig. 5C–D). For start returns, there were significant effects of Treatment, $F(1,53) = 30.8$, $p < 0.0001$, Day, $F(17,901) = 110.1$, $p < 0.0001$, Treatment $\times$ Day, $F(17,901) = 4.6$, $p < 0.0001$, Surgery $\times$ Day, $F(17,901) = 4.4$, $p < 0.001$, and Treatment $\times$ Surgery $\times$ Day, $F(17,901) = 2.1$, $p < 0.05$. Examination of Fig. 5E–F reveals that animals treated with MA (SHAM-MA or ADX-MA), regardless of Surgery, returned to the start more often beginning on day 5 compared to SAL-treated groups (SHAM-SAL or ADX-SAL). The interaction was the result of ADX-MA animals on Day 4 committing fewer start returns than SHAM-MA animals ($p < 0.05$).

ASR/PPI—There were no significant effects of Treatment, Surgery, or interactions between treatment and surgery on the startle response in the presence or absence of prepulse stimuli (not shown).

Monoamines—In the neostriatum, the main effect of Treatment was significant showing that the MA-treated groups had 35% decreased DA (Fig. 6A) and 34% decreased 5-HIAA (Fig. 6D), $F(1,38) = 15.5$ and 15.1 , respectively, $p < 0.001$. There were no significant effects on DOPAC (Fig. 6B) or 5-HT ($p < 0.08$; Fig. 6C). There were no significant main effects of Surgery for 5-HIAA levels ($p < 0.06$) or the other monoamines. None of the Treatment $\times$ Surgery interactions were significant although a trend was seen for 5-HT ($p < 0.07$). Direct comparison of SHAM-MA to ADX-MA groups demonstrated that the ADX-MA group had higher DA, 5-HT, and 5-HIAA levels compared to the SHAM-MA group, $t(18) = 2.6$, 2.9 , and 2.3 , respectively, all $p < 0.05$.

The main effect of MA treatment decreased hippocampal 5-HT (Fig 6E) and 5-HIAA (Fig. 6F) compared to the two control groups, Treatment, $F(1,39) = 19.1$ and 9.2 , respectively, $p < 0.001$. There were no significant main effects of Surgery although 5-HIAA showed a trend

($p < 0.07$). An inspection of the data suggested that the ADX-MA group had higher 5-HT and 5-HIAA levels than the SHAM-MA group, which was supported by direct comparison *t*-tests ($t(13) = 2.3$ and 2.4 , respectively, $p < 0.05$).

In the entorhinal cortex there was a Treatment main effect on 5-HT in which the MA-treated groups showed a 38% decrease in 5-HT, Treatment, $F(1,40) = 12.2$, $p < 0.01$ (Fig 6G), and 32% decreased levels of 5-HIAA, Treatment, $F(1,41) = 14.0$, $p < 0.001$ (Fig. 6H), compared to the control groups. Neither the main effect of Surgery (5-HT, $p < 0.08$; 5-HIAA, $p > 0.10$) nor the Treatment $\times$ Surgery interaction (both $p > 0.20$) was significant. There were no significant effects on DA levels. The mean $\pm$ SEM in pg/mg tissue for DA were: SHAM-SAL = 103 ± 8 ; SHAM-MA = 102 ± 22 ; ADX-SAL = 123 ± 26 ; ADX-MA = 112 ± 23 .

Discussion

In the present study we blocked the adrenal response to MA treatment to determine if the learning deficits observed previously would be blocked or attenuated (Herring et al., 2008). We also determined whether ADX altered the neurotoxic and hyperthermic effects of binge MA treatment. ADX did not change the magnitude or extent of water maze learning deficits (CWM performance in the absence of distal cues), the decreases in DA at 72 h, or the increases in GFAP at 72 h, even though the ADX-MA-treated animals showed no hyperthermia during treatment. It was noted too that ADX, regardless of treatment, had slightly higher 5-HT in some regions and whether this was the result of the absence of hyperthermia in the ADX groups is not known.

Hyperthermia is thought to be permissive for neurotoxicity to occur (Ali et al., 1994; Bowyer et al., 1992; Bowyer et al., 1994; Cappon et al., 1997) and the lack of protection on dopaminergic and GFAP markers of neurotoxicity at 72 h is noteworthy given that ADX-MA animals demonstrated essentially normal body temperatures while SHAM-MA animals displayed the typical pattern of hyperthermia during MA treatment. Previous studies have indicated that hyperthermia during MA exposure plays a role in the neurotoxic effects. For example, rats treated at an ambient temperature of 23°C with 4×5 mg/kg MA showed 60% decreased striatal DA 3 days after treatment, but only 30% decreased levels 14 days after treatment (Bowyer et al., 1992), however rats with core body temperatures $>41^{\circ}\text{C}$ had greater DA depletions than rats with core body temperatures $<41^{\circ}\text{C}$ (Bowyer et al., 1994). In the present experiment we intervened before body temperatures exceeded 41°C because past experience shows that this reduces mortality. Contrary to hyperthermic conditions, when MA is administered in a cold environment (4°C) and hypothermia is induced, only a transient 30% decrease in striatal DA levels are observed 3 days following MA treatment with levels similar to control animals 14 days post-treatment (Bowyer et al., 1992). Similarly, mice administered MA (4×10 mg/kg) show 80% depletions of striatal DA when dosed at 23°C , but only 20% reductions when dosed at 4°C (Ali et al., 1994). However, under certain conditions hyperthermia is not necessary to induce neuronal damage. For instance, MA-induced neurotoxicity was not prevented in mice given reserpine, a vesicular monoamine transporter (VMAT2) inhibitor and hypothermia-inducing agent (Albers and Sonsalla, 1995; Thomas et al., 2008). ADX has previously been shown to suppress the hyperthermic response to 1 mg/kg MA (Makisumi et al., 1998) and in the present experiment we show that ADX suppresses hyperthermia following a higher dose of MA (10 mg/kg $\times 4$). Unique to the present experiment is the fact that ADX-MA animals showed only a small and transient decrease in body temperature but still showed dopaminergic reductions and reactive astrogliosis 72 h later, essentially the same as those seen in the SHAM-MA animals. Makisumi et al. (1998) suggest that glucocorticoids are permissive for the hyperthermic response and MA (4×10 mg/kg) induces a marked increase in corticosterone that lasts for at least 3 days (Herring et al., 2008). Interestingly, we also noted

slightly higher 5-HT levels in the ADX groups an observation that may benefit from further investigation since it is known that the serotonergic system and corticosterone are linked (Fuller, 1992;Maines et al., 1998;Meijer et al., 1997). Taken together, the data suggest that hyperthermia and/or increased corticosterone levels may have a larger role in MA-induced serotonergic reductions than in dopaminergic reductions.

We have previously demonstrated that MA binge-treated animals exhibit greater than four-fold increases in CWM latency and errors compared to SAL-treated animals and show no evidence of performance catch-up after 15 days of testing (Herring et al., 2008). In the present experiment, we extended testing to 18 days and demonstrated that MA-treated animals did not catch-up to the SAL-treated groups' levels of performance for latencies, errors, and start returns even with three additional days of testing. Therefore, the magnitude and extent of the deficits observed in maze learning after MA treatment are unique compared to learning deficits utilizing other tasks (*cf.*, novel object recognition or MWM). While ADX-MA animals appeared to perform slightly better than the SHAM-MA animals, direct comparison between the groups showed no differences. Other psychostimulants, such as fenfluramine, 5-methoxy-diisopropyltryptamine (5-MEO-DIPT), and $\pm$ 3,4-methylenedioxymethamphetamine (MDMA), have also been shown to disrupt learning in the CWM but to a much lesser extent than seen with MA (Able et al., 2006;Skelton et al., 2004;Williams et al., 2002;Williams et al., 2007). However, no direct comparison is possible since those earlier experiments tested animals in the CWM under lighted conditions where distal cues could be used to help navigate through the maze, whereas in the previous binge MA experiment (Herring et al., 2008) and the present one, distal cues were eliminated by testing under infrared lighting, which we have shown makes the task much more difficult (Skelton et al., 2004;Williams et al., 2002;Williams et al., 2007). In a previous examination of the relationship between corticosterone and CWM deficits, we demonstrated that the smaller CWM deficits observed with fenfluramine were prevented if the increase in corticosterone produced by the drug was blocked pharmacologically (Skelton et al., 2004) or surgically (ADX) (Williams et al., 2002).

We demonstrated increased GFAP in the neostriatum and decreased monoamines at 72 h after MA treatment in both the neostriatum and hippocampus (Herring et al., 2008) as have others (Bowyer et al., 1994;Cappon et al., 1997;O'Callaghan and Miller, 2002;O'Dell and Marshall, 2002). The neostriatum has been implicated in sequence learning (Cook and Kesner, 1988;Potegal, 1972) and the CWM involves learning a sequence of turns to find the escape. Others have shown that binge MA exposure disrupts learning in a test of route-based motor learning (in which animals learned a specific path through corridors without choices) by showing longer latencies to complete the task or reductions in 'directness' to the goal (Chapman et al., 2001;Daberkow et al., 2005), but the exact relationship between sequence and egocentric learning is unknown. It is known that some types of egocentric learning depend on head-direction cells in the presubiculum, grid cells in the entorhinal cortex, and other regions (Fuhs and Touretzky, 2006;McNaughton et al., 2006;Rondi-Reig et al., 2006;Sargolini et al., 2006;Whishaw et al., 1997;Witter and Moser, 2006). Loss of 5-HT has been implicated in a variety of specific human and animal cognitive deficits (Chudasama and Robbins, 2006;Schmitt et al., 2006), thus the depletions in 5-HT and 5-HIAA in the entorhinal cortex may be involved in MA-induced CWM deficits. Taken together, the serotonergic system should be an area of focus in future research on MA-induced learning and memory deficits.

Acutely, the binge dosing regimen used here increased stereotypic behavior in the MA groups during the first 24 h post-treatment an effect that was gone by 30 h. The ADX-MA group showed a small but significantly higher stereotypy score over this period than did the

SHAM-MA group, however given how small the difference was it does not suggest a meaningful difference as a result of the ADX surgery.

One concern with using ADX is that the learning ability of the animals may be affected, however examination of Figure 5 shows that ADX-SAL animals performed as well as SHAM-SAL animals, a finding we have previously observed in both the MWM and CWM (Williams et al., 2002). This is an interesting finding in that intermediate levels of corticosterone have been shown in other studies to augment spatial learning and memory, but very low or high levels have been shown to be detrimental (Lupien and McEwen, 1997). It is possible that the short interval between ADX and behavioral testing and/or the different cognitive tests utilized here resulted in the lack of learning deficits observed between intact and ADX animals.

In this experiment, we did not find evidence of novel object recognition deficits, although these have been reported previously after binge MA treatment (Belcher et al., 2005; Belcher et al., 2006; Belcher et al., 2008; Bisagno et al., 2002; He et al., 2006; Herring et al., 2008; Schroder et al., 2003). Finding no novel object recognition effects is similar to recently reported data (Clark et al., 2007). Another recent study showed that if MA-treated animals are compared to SAL-treated animals for time spent with the novel object, no differences were obtained, but if the amount of time spent with the familiar object was used to adjust retention performance then differences were seen (Belcher et al., 2008). This suggests that MA-related novel object recognition differences may be sensitive to time spent with the familiar object when familiarization times are not equal.

We also found that MA had no effects on elevated zero maze performance. Previous studies examining anxiety-related behavior after MA have reported mixed results. Low dose MA appears to increase the amount of time spent in the open arms of the elevated plus maze 20 min following injection (Szumlinski et al., 2001). Chronic (20 mg/kg, 5 days) MA treatment has been reported to increase the time spent in open arms ~2 weeks after the last treatment (He et al., 2005); however, another study comparing chronic (2 mg/kg, 7 days) and single (4 mg/kg) MA treatment revealed a preference for closed arms 3 and 5 days following the last dose (Hayase et al., 2005). In addition, no effects of MA on sensory gating (prepulse inhibition of the acoustic startle response) were observed in the present experiment. To the extent that sensory gating differences might interfere with learning, no evidence for such effects was obtained.

The present results indicate that the deficits in egocentric learning are not attributable to the increase in adrenal output during or after MA exposure. In addition, hyperthermia does not appear to be required for egocentric water maze learning impairments or for dopaminergic or serotonergic reductions after MA exposure. Nor did the significantly greater DA or 5-HT neostriatal or hippocampal 5-HT recovery seen in the ADX-MA vs. SHAM-MA groups make any significant difference in CWM performance. Given this, the robust deficits observed in MA-treated animals in egocentric water maze learning may provide a useful model for investigations of the mechanisms underlying the cognitive deficits reported in chronic and/or abstinent MA users (Barr et al., 2006; Meredith et al., 2005).

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Figure 1.

The body temperatures of animals in Experiment 1 (A & B) and Experiment 2 (C & D). No differences in initial temperatures were observed; however, MA produced significant increases in body temperature in SHAM animals starting 30 min after the first dose (A). However, hypothermia was observed from 30–90 min in ADX-MA animals and a slight increase in temperature at 510 min (B). Exp. 2 revealed similar findings: SHAM-MA animals demonstrated hyperthermia beginning at 30 min (C) while ADX-MA did not (D). The arrows denote the times when MA was delivered. * $p < 0.05$.

Figure 2. Monoamine levels in neostriatum (A–D), hippocampus (E–F), and entorhinal cortex (G–H) 3 days following MA exposure. MA-treated animals regardless of adrenalectomy demonstrated decreased levels of DA (A), DOPAC (B) in the neostriatum and 5-HT (C, E & G) and 5-HIAA (D, F, & H) in all regions. In addition, by t-test, the ADX-MA animals had higher levels of 5-HT and 5-HIAA in the neostriatum, and 5-HIAA in the hippocampus compared to SHAM-MA animals. Group sizes: SHAM SAL = 6–10; SHAM MA = 5–6; ADX-SAL = 6–8; ADX-MA = 6–9). * $p \leq 0.05$ MA vs SAL, # $p < 0.05$ ADX-MA vs SHAM-MA.

Figure 3. GFAP levels 3 days following MA exposure by western blot analysis. GFAP was increased in MA-treated animals regardless of regimen at 72 h following the first dose compared to SAL-treated animals. Group sizes: SHAM-SAL = 8, SHAM-MA = 6, ADX-SAL = 8, ADX-MA = 7). * $p < 0.05$ MA vs SAL.

Figure 4.

Mean ($\pm$ SEM) stereotypy scores 6–30 h following the first dose of MA. MA-treated groups regardless of surgical condition displayed increased stereotypy compared to SAL-treated groups from the first scoring period out to 24 h post-treatment. Differences were essentially gone by 30 h post-treatment. Group sizes: SHAM -SAL = 16, SHAM-MA = 17, ADX-SAL = 14, ADX-MA = 15). $**p \leq 0.05$ ADX-MA vs SHAM-MA; both MA groups differed significantly from both SAL groups (not marked).

Figure 5.

CWM performance beginning 2 weeks following MA treatment. SHAM-treated animals are displayed in A, C, E and ADX-treated animals are displayed in B, D, F. MA-treated animals, regardless of ADX, demonstrated decreased latencies beginning on day 6 (A, B), increased number of errors beginning on day 5 (C, D), and increased start returns beginning on day 5 (E, F). On day 4 ADX-SAL animals committed fewer start returns than SHAM-SAL animals (E,F). Group sizes: SHAM-SAL = 15, SHAM-MA = 17, ADX-SAL = 12, ADX-MA = 13. * $p \leq 0.05$ MA *vs* SAL, # $p \leq 0.05$ ADX-MA *vs* SHAM-MA.

Figure 6. Monoamine levels in neostriatum (A–D), hippocampus (E–F), and entorhinal cortex (G–H) ~35 days following MA exposure. MA-treated animals regardless of ADX demonstrated decreased levels of DA (A) but no effects on DOPAC (B) in the neostriatum. There was a trend for decreased 5-HT (C) and 5-HIAA (D) in the neostriatum. MA-treated animals regardless of ADX demonstrated decreased levels of 5-HT (E, G) and 5-HIAA (F, H) in the hippocampus and entorhinal cortex, respectively. In addition, ADX animals displayed increased 5-HIAA compared to SHAM animals in the hippocampus. Analysis of just the MA-treated animals by t-test showed that in the neostriatum and hippocampus the levels of monoamines were greater in ADX-MA relative to SHAM-MA with the exception of DOPAC in the neostriatum. Group sizes: SHAM-SAL = 10–11, SHAM MA = 9–11, ADX-SAL = 9–10, ADX-MA = 10–11). * $p \leq 0.05$ MA vs SAL, # $p < 0.05$ ADX-MA vs SHAM-MA.