Published in final edited form as:

Synapse. 2011 May ; 65(5): 368–378. doi:10.1002/syn.20854.

Comparison of (+)-methamphetamine, $\pm$ -methylenedioxymethamphetamine (MDMA), (+)-amphetamine and $\pm$ -fenfluramine in rats on egocentric learning in the Cincinnati water maze

Charles V. Vorhees^{1,*}, Elizabeth He², Matthew R. Skelton¹, Devon L. Graham¹, Tori L. Schaefer¹, Curtis E. Grace³, Amanda A. Braun⁴, Robyn Amos-Kroohs³, and Michael T. Williams¹

¹ Division of Neurology, Department of Pediatrics, Cincinnati Children's Research Foundation and University of Cincinnati College of Medicine, Cincinnati, OH 45229

² University of Cincinnati College of Medicine Medical School Program, Cincinnati, OH 45267

³ Graduate Program in Molecular and Developmental Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45229

⁴ Graduate Program in Neuroscience, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Abstract

(+)-Methamphetamine (MA), ($\pm$)-3,4-methylenedioxymethamphetamine (MDMA), (+)-amphetamine (AMPH), and ($\pm$)-fenfluramine (FEN) are phenylethylamines with CNS effects. At higher doses, each induces protracted reductions in brain dopamine and/or serotonin. Chronic MA and MDMA users show persistent monoamine reductions and cognitive impairments. In rats, similar neurochemical effects can be induced, yet cognitive impairments have been difficult to demonstrate. We recently showed that rats treated on a single day with MA (10 mg/kg $\times$ 4 at 2 h intervals) exhibit impaired egocentric learning (Cincinnati water maze; CWM) without affecting spatial learning (Morris water maze) (Herring et al., 2008). Whether this effect is unique to MA or is a general characteristic of these drugs is unknown. Accordingly, this experiment compared these drugs on CWM performance. Drugs were given s.c. in four doses at 2 h intervals. MA doses were 10 or 12.5 mg/kg/dose, AMPH 25 mg/kg/dose (to match MA12.5-induced hyperthermia), MDMA 15 mg/kg/dose (previously established hyperthermia-inducing dose), and FEN 16.5 mg/kg/dose (equimolar to MA12.5). Two weeks later, rats were tested in the CWM (2 trials/day, 21 days). AMPH and MA (both doses) induced significant increases in CWM errors and latency to reach the goal with no differences in swim speed. MDMA and FEN did not significantly alter learning. Given that FEN selectively and MDMA preferentially affect serotonin whereas AMPH selectively and MA preferentially affect dopamine, the data suggest that egocentric learning may be predominantly dopaminergically-mediated.

*Correspondence: Charles V. Vorhees, Ph.D., Cincinnati Children's Research Foundation, Division of Neurology (MLC 7044), 3333 Burnet Ave., Cincinnati, OH 45229-3039, Tel. 513-636-8622, Fax 513-636-3912, charles.vorhees@cchmc.org.

Keywords

Methamphetamine; 3,4-methylenedioxymethamphetamine; MDMA; d-amphetamine; fenfluramine; maze learning; swimming maze; Cincinnati water maze; egocentric learning; hyperthermia; route-based learning; rat

Introduction

Methamphetamine (MA) is an addictive stimulant that has become increasingly popular (EMCDDA, 2007; Johnston et al., 2008; Johnston et al., 2009). Chronic use of MA results in long-lasting neurochemical and cognitive alterations (Baicy and London, 2007; Barr et al., 2006; Chang et al., 2007; Meredith et al., 2005). These deficits consist of impairments in working memory, attention, and executive function even after extended periods of abstinence (London et al., 2004; Monterosso et al., 2005; Salo et al., 2002; Salo et al., 2005; Salo et al., 2007; Thompson et al., 2004; Volkow et al., 2001a; Volkow et al., 2001b). The mechanisms of these changes are not understood. Autopsy and neuroimaging studies show reductions in brain monoamines and reuptake transporters in chronic MA users (Baicy and London, 2007; Barr et al., 2006; Chang et al., 2007; Meredith et al., 2005), but whether these are the changes resulting in the cognitive deficits is unclear. Similar cognitive effects have been reported in chronic 3,4-methylenedioxymethamphetamine (MDMA) abusers (Bolla et al., 1998; Gouzoulis-Mayfrank et al., 2000; Halpern et al., 2004; Jacobsen et al., 2004; Kalechstein et al., 2007; McCann et al., 1994; McCann et al., 1999).

As a model of MA-induced cognitive deficits, we previously showed that adult rats receiving a neurotoxic regimen of MA (10 mg/kg 4 times at 2 h intervals) exhibit deficits in egocentric learning in the Cincinnati water maze (CWM) under infrared lighting, i.e., in the absence of distal cues (Herring et al., 2008). More recently, we have replicated and extended this finding (Herring et al., 2010). We and others have also reported MA-induced impairments in novel object recognition (Belcher et al., 2005; Bisagno et al., 2002; He et al., 2006; Herring et al., 2008; Schroder et al., 2003). By contrast, we and others fail to find allocentric learning deficits in the Morris water maze (MWM) (Friedman et al., 1998; Herring et al., 2008; Schroder et al., 2003).

Experiments on another substituted amphetamine, fenfluramine (FEN), show that it too induces CWM deficits but under different test conditions and these deficits were prevented by prior adrenalectomy (ADX) or corticosterone synthesis inhibition (Skelton et al., 2004; Williams et al., 2002). By contrast, ADX has no effect on MA-induced CWM deficits when tested under infrared lighting (Herring et al., 2010). FEN-treated rats (15 mg/kg $\times$ 4 at 2 h intervals) were tested under visible red light.

Another substituted amphetamine of abuse, MDMA, also induces learning deficits. MDMA induces deficits in both the MWM (Able et al., 2006; Sprague et al., 2003) and CWM, the latter when tested under low-level visible light after 15 mg/kg $\times$ 4 at 2 hr intervals on a single day (Able et al., 2006).

The prototype of this group of compounds is amphetamine (AMPH). Unfortunately, there is a gap in knowledge concerning the cognitive effects of AMPH when given at monoamine-depleting doses and we have no data on the effects of AMPH on CWM performance.

Each of these drugs has overlapping but different effects on brain monoamines, transporters, and synthetic enzymes. AMPH is selective for dopaminergic neurons (Wagner et al., 1980), the dopamine transporter (DAT) (Krasnova et al., 2001; Scheffel et al., 1996), and tyrosine

hydroxylase (TH) (Ellison et al., 1978;Schmitz et al., 2001;Sulzer et al., 1993;Sulzer et al., 2005) with little or no affinity for serotonergic neurons (Belcher et al., 2005;Peat et al., 1985), the serotonin (5-HT) transporter (SERT) (Belcher et al., 2005;Han and Gu, 2006), or tryptophan hydroxylase (TPH) (Peat et al., 1985). MA affects the dopaminergic (DA) and serotonergic systems (Ricaurte et al., 1980;Shoblock et al., 2003a;Shoblock et al., 2003b). In the striatum, it affects DA, DAT, and TH more than 5-HT (Finnegan and Karler, 1992;Wallace et al., 2001), SERT (Kokoshka et al., 1998), or TPH (Hotchkiss and Gibb, 1980); however effects on the serotonergic system can be greater in other regions (Haughey et al., 1999;Herring et al., 2008;Ricaurte et al., 1980). MDMA also affects both DA and 5-HT neurons, but affects 5-HT, SERT, and TPH more than DA, DAT, and TH (Wallace et al., 2001). FEN is selective for 5-HT, SERT, and TPH with no effect on DA, DAT, or TH (Colado et al., 1993;Harvey and McMaster, 1975;Kleven et al., 1988;Malberg and Seiden, 1997;Rothman et al., 1999;Zacsek et al., 1990). Hence, comparing these drugs for their cognitive effects may provide insight into how their differing mechanisms contribute to egocentric learning.

We previously demonstrated that MA impairs CWM egocentric learning while sparing MWM allocentric learning (Herring et al., 2008;Herring et al., 2010). How does wayfinding by egocentric and allocentric navigation differ? Allocentric learning has been widely investigated and is a form of hippocampally-dependent spatial navigation or mapping that relies on distal cues (D'Hooge and De Deyn, 2001) and is an aspect of declarative and/or episodic memory (memory for people, places, facts, and events). However, allocentric navigation is only one form of wayfinding. When animals do not have distal cues with which to pilot to a goal they can still successfully forage for food and return to their home using other cues. Egocentric learning relies upon proprioceptive self-movement cues, velocity, changes in direction, and signposts (i.e., landmarks that demarcate a junction rather than an orientation). Just as place cells have been identified in the hippocampus that fire as a function of spatial position, cells in other brain regions have been identified that fire in response to head orientation. Head direction cells connect to grid and border cells in the entorhinal cortex and other regions providing a sense of direction (Fuhs and Touretzky, 2006;McNaughton et al., 2006;Rondi-Reig et al., 2006;Sargolini et al., 2006;Solstad et al., 2008;Whishaw et al., 1997;Witter and Moser, 2006).

There are subtypes of egocentric learning: (a) route-based and (b) path integration. Route-based learning requires paths and junctions (or nodes), usually forming a lattice or grid (Byrne, 1982). These grids are found in virtually all mammalian species that forage and find their way back to a home base and are highly regularized in non-human primates (Di and Suarez, 2007). The same ability is found in humans. This form of navigation is often distinguished from path integration (Benhamou, 1997;Etienne and Jeffery, 2004;Whishaw, 1998) in which an animal leaves its home and forages in multiple locations, but in returning home neither retraces its route nor travels back along grid lines by way of interconnected nodes. Instead, it takes a direct path home across territory not necessarily taken before, thereby forging a new direction based on 'dead-reckoning' of the most direct route. This ability is based on the principle of vector addition, i.e., a combination of distances and angles and is well known in humans (Shrager et al., 2008;Wolbers et al., 2007). It is not known whether the CWM is a test of route-based or path integration, or a combination of both. However, by testing animals in the dark, distal cues (required for allocentric navigation) are eliminated. This also eliminates signposts and visual 'flow' (from moving past the surrounding environment) which are used in path integration. What this leaves at the animal's disposal are proprioceptive cues of limb movements, tactile cues, vestibular cues that respond to acceleration and orientation, detection of environmental surfaces by touch, and olfactory cues. Given that the CWM does not permit short-cut or direct paths to the goal, it appears to be a route-based task.

The present experiment was designed to compare these four amphetamine congeners for their effects on route-based learning to determine if their previously established monoaminergic effects exhibit a systematic relationship to CWM performance when tested under identical environmental conditions (infrared light to eliminate distal cues), identical treatment conditions (same ambient temperature, same limit on peak temperature, and same method of measuring body temperature), and identical test procedures (same delay interval between day of treatment and the start of testing, and same number of trials/day and days of testing). We hypothesized that the drugs with the most severe dopaminergic effects would have the greatest effects on this form of egocentric learning.

Materials and Methods

Animals

Subjects were adult male Sprague-Dawley CD IGS rats (325–350 g) from Charles River Laboratories, Raleigh, NC. Rats were acclimated to the vivarium (temperature, $19 \pm 1^\circ\text{C}$, $50 \pm 10\%$ humidity; 14 h light:10 h dark cycle (lights on at 600 h) for 1–2 weeks prior to drug treatment, housed in pairs in cages measuring $46 \times 24 \times 20$ cm, separated into same-sized cages 7 days before drug treatment, and individually housed thereafter (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.

Drugs

(+)-Methamphetamine HCl and ($\pm$)-3,4-methylenedioxymethamphetamine HCl were obtained from the National Institute on Drug Abuse (Rockville, MD). ($\pm$)-Fenfluramine HCl and (+)-amphetamine hemi- SO_4 were obtained from Sigma (Sigma-Aldrich, St. Louis, MO). All doses are expressed as the free base. All doses were administered subcutaneously in a dosing volume of 3 ml/kg dissolved in saline or saline-alone to controls (Saline group). All drugs were given 4 times on a single day at 2 h intervals starting at 900 h.

Drug Treatments

MA was given at two dose levels. One group received MA at 10 mg/kg/dose (MA10). This is a known neurotoxic dose that we and many others have used to produce monoamine reductions (Bowyer et al., 1992; Bowyer et al., 1994; Broening et al., 1997; Broening et al., 2005; Cappon et al., 1996; Haughey et al., 2000; Kokoshka et al., 2000; Metzger et al., 2000; Pu et al., 1994; Pu et al., 1996; Pu and Vorhees, 1994; Pu and Vorhees, 1995; Riddle et al., 2002; Ugarte et al., 2003), as well as GFAP increases and other evidence of neurotoxicity (Bowyer and Holson, 1995; O'Callaghan and Miller, 2002). It is also the dose of MA we used previously that produced CWM deficits when tested under infrared lighting conditions (Herring et al., 2008; Herring et al., 2010). In order to determine whether the maze deficits might be worse at higher doses, we included a second MA group. For this, we selected 12.5 mg/kg/dose of MA (MA12.5). MDMA was given at a dose of 15 mg/kg/dose (MDMA). This was based on a prior experiment in which we showed that this dose induced CWM effects when tested under visible lighting (Able et al., 2006) and is a dose known to reduce 5-HT, SERT, and TPH, e.g. (Haughey et al., 2000).

Fenfluramine (FEN) was given at 16.5 mg/kg/dose. This is an equimolar dose to the MA12.5 group, and similar to the dose that induced CWM deficits previously when tested under visible light (Williams et al., 2002). The change in dose for FEN was based in part on how it affects body temperature. The effects of FEN on body temperature are dependent on both ambient temperature and dose. Raising ambient temperature shifts the body temperature response from inducing hypothermia at an ambient temperature of 21°C to

hyperthermia at an ambient temperature of 32°C. Since the ambient temperature used in the present experiment was 23°C (see below), the increased dose reduced the hypothermic effect of the drug compared to our previous experiment (Williams et al., 2002). This reduced the outside chance that a learning effect might be caused by hypothermia per se (see below).

We had no prior experience with AMPH in the CWM. Because of this, we selected a dose based on the degree of drug-induced hyperthermia. For this, we chose a dose that induced the same degree of initial hyperthermia as the MA12.5 dose group which we determined empirically. This dose was 25 mg/kg/dose.

Groups

Twenty-one rats were assigned to each of 5 treatment groups: SAL, MA10, MA12.5, MDMA, FEN, or AMPH (N = 105 total) and dosed with 4 doses of the designated drug as described above on a single day. The study was conducted in three cohorts balanced by group and spaced 2–3 weeks apart, i.e., 7/group/cohort. Core body temperature was monitored prior to the first dose, during dosing (6 h at 15 min intervals), and for 4 h after the last dose every 15 min. Food was reintroduced after the final temperature reading. Maze testing commenced 2 weeks post-drug treatment as in our previous experiment with MA. The study was conducted in three cohorts with 7 animals per group per cohort. Ambient room temperature during dosing for all three cohorts was $23.1 \pm 1^\circ\text{C}$.

Body Temperature Monitoring

Body temperature was measured with subcutaneously implanted temperature transponders (IPTT-300: Bio Medic Data Systems, Seaford, DE) that were inserted under light isoflurane anesthesia below the skin in the dorsum 3 days prior to treatment. Transponders have the advantage of preventing the documented stress of handling and rectal temperature measuring methods (Bae et al., 2007; Balcombe et al., 2004). Animals were cooled in a shallow water bath if transponder readings reached 40.2°C (Herring et al., 2008) and were maintained in the bath until a temperature reading was obtained that was below 40°C. While the animal was in the bath, body temperatures were monitored every 5 min.

Straight channel swimming

One day prior to CWM testing (day 13 after drug 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/trial (Vorhees et al., 2008). Straight channel swimming served three functions: (a) to acclimate animals to swimming, (b) to teach that escape was possible by climbing on a submerged platform at the opposite end of the channel, and (c) to determine if all animals had comparable swimming ability by timing their transit time from the start to the platform on each trial.

Cincinnati Water Maze

CWM performance was tested for 21 days beginning 14 days after treatment using procedures previously described (Vorhees et al., 2008). Animals must swim through a 9-unit multiple T-maze to locate a submerged escape platform. Animals were tested under complete darkness (using infrared LED emitters) in order to eliminate distal visual cues. This procedure requires animals to rely on egocentric cues to find the goal. Two trials/day (5 min limit/trial) were given with an intertrial interval of not less than 5 min if they reached the trial limit. Animals reaching the time limit were not shown the route to the escape but were removed from the maze immediately. Latency to escape and number of T, stem, and start return errors were recorded separately on each trial. An error was defined as head and shoulder entry in a stem or arm of a T that was not on the path to the goal. On early trials,

many animals failed to find the escape within the time limit but succeeded after several days. A few animals took longer to learn the path and sometimes these animals would stop searching and remain in one T for extended intervals. In order to correct for such search failures, these animals were given a score equal to that of the animal making the most errors within the 5 min time limit +1.

Statistical analyses

Data for body temperature and performance in the CWM were analyzed by mixed linear analyses of variance (ANOVA) in which treatment was a between factor and time or day a repeated measure factor. Variance-covariance matrices were examined for best fit to structural models and the model of best fit was employed. Degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997). Where significant interactions were obtained, slice-effect ANOVAs were performed at each level of the repeated measure factor. Where these were significant, pairwise group comparisons were conducted using the Hochberg step-up method. Since previous data showed that MA, MDMA, and FEN impair CWM performance, unidirectional tests were used based on the *a priori* prediction that these groups would be impaired (i.e., that none of the drugs would improve performance). Significance was set at $p \leq 0.05$.

Results

Mortality

There was no mortality in the Saline (0/21), MA10 (0/21) or FEN (0/21) groups. However, 3 animals in the MA12.5 group died (3/21), leaving 18 for testing. In the AMPH group 5 died (5/21), leaving 16 for testing.

Body Temperature

The effects of the drugs on body temperature are shown in Fig. 1. As can be seen, all 4 drugs affected body temperature. There was a significant Treatment main effect, $F(5,117) = 53.9$, $p < 0.0001$, a Time main effect, $F(32,3343) = 3.6$, $p < 0.0001$, and a Treatment $\times$ Time interaction, $F(160,3444) = 3.0$, $p < 0.0001$. Slice-effect ANOVAs at each time showed Treatment effects at all intervals ($p < 0.001$). *A posteriori* comparisons of each drug group to Saline controls showed that the MA10, MA12.5, and AMPH groups were hyperthermic from the second interval to the last (bracketed range at top of Fig. 1). MDMA also induced hyperthermia, but with a delayed onset that was significant from the second dose (2 h) onward (bracketed range in middle of Fig. 1). FEN, on the other hand, induced hypothermia that was significant from 30–300 min and at two later intervals (bracketed range at bottom of Fig. 1 plus two later intervals shown by asterisks).

Note that the hyperthermic pattern, in terms of rise, peak, and duration seen in the AMPH group followed that of the MA12.5 group after the first and second doses, but decreased compared to the MA12.5 and MA10 groups at and after the third and fourth doses.

In order to understand the impact of the cooling intervention, we tallied the number of animals that received cooling interventions, which occurred when a body temperature reached or exceeded 40.2°C. The number of animals cooled per group was as follows: Saline = 0/21, MA10 = 10/20 (1 animal had a nonfunctioning temperature transponder), MA12.5 = 11/18, AMPH = 8/16, MDMA = 7/21, FEN = 0/21. We then compared whether the cooled vs. un-cooled subgroups differed significantly in maze performance (see below).

Body Weight

Body weight data are shown in Table 1. Body weight was measured prior to treatment, at 8 and 72 h after the first dose, and at one week intervals thereafter. An ANOVA on pre-treatment body weights showed no significant differences. At 8 h after the first dose (i.e., 2 h after the last dose), there was a significant Treatment effect, $F(5,474) = 2.57$, $p < 0.05$, however, *a posteriori* comparisons between the Saline group and each drug group failed to reach significance (not shown). The Treatment effect was also significant 72 h post-treatment, $F(5,466) = 5.58$, $p < 0.0001$. *A posteriori* comparisons at this time point showed that each group differed from Saline (Table 1). A repeated measure ANOVA on weekly body weight showed no significant Treatment main effect, however the Treatment $\times$ Week interaction was significant, $F(15,336) = 3.02$, $p < 0.0001$, as was the main effect of Week, $F(3,336) = 402.32$, $p < .0001$. Slice ANOVAs performed on each week showed no significant treatment effect on any week, hence, the interaction was not the product of any one week difference but rather the change over weeks between groups. For example, the Saline group did not lose weight following treatment and between weeks 1 and 4 gained an additional 41 g on average. By contrast, the drug groups each lost weight after treatment and the groups that lost the largest amount, also gained the largest amount between weeks 1 and 4. For example, the MA10, FEN and MDMA groups all lost about the same amount of weight following treatment and each gained 52–56 g between week 1 and 4. This as compared to groups MA12.5 and AMPH which lost about 10 g more than the other drug groups following treatment and gained more (69–71 g) between week 1 and 4. The net result was that all groups ended statistically equal in body weight but the rate of weight gain was different among the groups. Body weights at 2 and 4 weeks are shown. The 2 week point is presented because it is the day on which CWM testing began and 4 week point is shown because it is within 24 h of the last day of testing.

Straight Channel Swimming

There was no Treatment main effect on straight channel swimming times. The main effect of Trial was significant, $F(3,336) = 46.2$, $p < 0.0001$. There was also a significant Treatment $\times$ Trial interaction, $F(15,336) = 1.7$, $p < 0.05$. Slice-effect ANOVAs showed a significant effect on trial-1 ($p < 0.001$) but not on trials 2-4 (F -values < 1). This effect was attributable to the FEN group. On Trial-1 the FEN group swam faster (shorter times) than the Saline group: Trial-1 mean $\pm$ SEM for Saline = 35.9 ± 3.1 s vs. FEN = 23.0 ± 3.0 s. Although the reason for this isolated effect is not known, it clearly does not reflect a deficit in swimming ability and disappeared after trial-1.

Cincinnati Water Maze

Multiple measures of CWM performance were recorded and intercorrelations among them were determined. An error in which an animal entered the left or right arm of a dead-end T was termed a T error, which is listed in Table 1 as “T”; an error in which an animal entered the stem leading to a dead-end T was termed a stem error or “S” in Table 2; an error in which an animal left and then returned to the start arm was termed as start return error or “R” in Table 2. Because we have collected data for a number of years combining T and R errors, we summed these as T+R in Table 2. Combining all three types of errors (T+S+R) was termed “total errors” (Tot err). Latency (s) was the time it took the animal to find the goal after being placed in the start position. Averaging each dependent measure across days provided an index of total performance on that variable, and Pearson correlation coefficients were calculated for each dependent variable to every other variable with the results shown in Table 2. On the first few days of testing, most animals fail to find the escape within the time limit but continue to search throughout the 5 min. Table 2A shows the intercorrelations with scores corrected as described in Methods. Table 2B shows the intercorrelations among variables with no correction for non-searching episodes.

As can be seen, no matter how the data are viewed, corrected or uncorrected, the correlations among the variables were high. In general, correlations were slightly higher with corrected scores than with uncorrected scores. Nevertheless, the correlations are sufficiently high that total error scores capture maze performance and it would be redundant to present each variable separately, therefore, only total error data are presented.

Total errors as a function of day are shown in Fig. 2 and averaged across days in Fig. 3. Because of the number of groups, Fig. 2A shows the Saline vs. AMPH group, and Fig. 2B shows Saline vs. MA12.5 and MA10 groups, and Fig. 2C shows Saline vs. the MDMA and FEN groups. There was a significant Treatment main effect on total errors, $F(5,114) = 5.1$, $p < 0.001$. The Day main effect was also significant, $F(20,2044) = 117.6$, $p < 0.0001$, whereas the Treatment $\times$ Day interaction was not, $F(100,2142) = 1.2$, $p < 0.08$. Since the Treatment $\times$ Day interaction was not significant, only the main effect of Treatment group was analyzed further by *a posteriori* pairwise comparisons (Fig. 3). Averaged across days, treatment group comparisons showed that the AMPH group made the most errors ($p < 0.01$). In addition, the MA12.5 and MA10 groups made significantly more errors than Saline controls ($p < 0.05$). The two MA groups did not differ from one another. Although both the FEN and MDMA groups made more errors than Saline controls, neither of these groups differed significantly from Saline controls.

Latency results showed the same pattern of group differences as did errors, i.e., there was a significant main effect of Treatment, $F(5,113) = 4.7$, $p < 0.001$. The main effect of Day was also significant, $F(20,2056) = 122.5$, $p < 0.0001$, whereas the Treatment $\times$ Day interaction was not. Treatment group averages ($\pm$ SEM, s) were: SAL = 105.8 ± 8.8 ; AMPH = 164.8 ± 10.1 ; MA12.5 = 134.3 ± 9.5 ; MA10 = 133.3 ± 8.8 ; MDMA = 117.4 ± 8.8 ; and FEN = 116.6 ± 8.8 .

3.5 Cooled Vs. Un-cooled Comparisons

Approximately half of the animals in the AMPH, MA10, MA12.5, and MDMA groups received cooling to prevent life-threatening hyperthermia. We conducted separate ANOVAs on CWM total errors for each of the four drug groups that induced hyperthermia with Cooling as the main effect. For AMPH, no significant main effect of Cooling ($F < 1$) or Cooling $\times$ Day interaction was found ($p = 0.18$). For MA12.5 and MA10, there was neither a significant main effect of Cooling ($Fs < 1$) nor Cooling $\times$ Day interaction ($Fs < 1$). Similarly, for MDMA, neither the main effect of Cooling ($F < 1$) nor the Cooling $\times$ Day interaction ($F < 1$) was significant. These data are summarized in Table 3.

Discussion

We compared four amphetamine drugs, three of which are widely abused psychostimulants, for their effects on swimming ability and egocentric learning. The three drugs that are psychostimulants (MA, AMPH, and MDMA) are widely taken drugs of abuse that cause hyperthermia and long-term monoamine reductions when taken in high doses (Green et al., 2003; O'Callaghan and Miller, 2002). In chronic stimulant users all three, and especially MA and MDMA, have been associated with residual cognitive impairments; for MA (Monterosso et al., 2005; Salo et al., 2002; Salo et al., 2005; Salo et al., 2007; Thompson et al., 2004; Volkow et al., 2001a; Volkow et al., 2001b), reviewed in (Barr et al., 2006; Meredith et al., 2005; Nordahl et al., 2003; Yoshida, 1997) and for MDMA (Bolla et al., 1998; Gouzoulis-Mayfrank et al., 2000; Halpern et al., 2004; Jacobsen et al., 2004; McCann et al., 1994; McCann et al., 1999), reviewed in (Kalechstein et al., 2007). The fourth drug, FEN, was included even though it is not a drug of abuse, because it has been shown to induce long-lasting 5-HT reductions. Clinically, FEN is not a psychostimulant, but rather an anorectic agent used for short-term appetite suppression.

We hypothesized that comparing the drugs for their effects on egocentric learning would provide indirect evidence as to which neurotransmitters are most likely to be involved given that each one affects dopaminergic and serotonergic systems differently. AMPH induced the largest impairments in CWM performance without affecting swim speed in pre-maze straight channel trials, indicating that the effect is on learning rather than swimming ability or motivation. It was also given at the highest dose because the dose selected was chosen to induce an initial hyperthermia response comparable to that seen after MA treatment. Certainly there are alternate ways of selecting doses for comparative analyses and we do not suggest that matching for initial hyperthermia is more than one of many possible ways substituted phenylethylamine doses could be compared. A similar but lesser impairment was seen after MA at both doses, but again the doses were lower and it may be that in the present context quantitative comparisons are of limited value, only whether an effect was obtained, and both doses of MA were effective at impairing learning two weeks after treatment. The results are of interest in part because AMPH reduces DA, DAT, and TH without affecting 5-HT, SERT, or TPH and MA affects DA, DAT, and TH preferentially in some regions of the brain. This is salient because the medial neostriatum has been implicated in egocentric learning (Anguiano-Rodriguez et al., 2007; Cook and Kesner, 1988; Potegal, 1972) and AMPH and MA have pronounced effects on neostriatal DA, DAT, and TH even though neurotransmitter, transporter, and/or enzymatic activity were not measured in this experiment.

By contrast, FEN reduces 5-HT, SERT, and TPH without affecting DA, DAT, or TH, and MDMA preferentially affect 5-HT, SERT, and TPH, and neither of these drugs had significant effects on CWM learning at the doses used here. These data suggest that the long-term cognitive effects of monoamine-depleting doses of amphetamine-like drugs on egocentric learning may be mediated dopaminergically rather than serotonergically (see also (Anguiano-Rodriguez et al., 2007)). However, if egocentric learning is tested under visible light, 5-HT can influence this form of learning, since neostriatal 5-HT reductions disrupt vision-dependent egocentric learning (Anguiano-Rodriguez et al., 2007). This is consistent with data that visual fields have inputs to the postsubiculum and retrosplenial cortex which in turn have inputs to head direction cells located in the anterodorsal nucleus of the thalamus (Clark et al., 2010). This is also consistent with previous data with FEN and MDMA administration that showed deficits in the CWM when examined under visible light conditions (Able et al., 2006; Skelton et al., 2004; Skelton et al., 2008; Williams et al., 2002).

Although consistent with our data, the idea that egocentric learning is mediated or influenced by dopaminergic systems should be interpreted cautiously because of limitations of the experiment. These include: (1) only one dose was tested of each drug (except MA); a more complete dose-response range would likely provide better evidence; (2) the doses were not matched on a mg/kg basis or molar basis, so inferences about relative magnitude of effect among the drugs is not possible; (3) despite efforts to match temperature profiles for MA and AMPH, core body temperatures did not remain comparable throughout the course of treatment and it is possible that these differences contributed to differences in outcome, although it is hard to believe that the lower temperatures in the AMPH animals exacerbated the learning deficits; (4) we did not have separate groups of animals on which brain monoamine changes were measured at a point when behavioral testing began, hence the relative effects on monoamine levels between drug groups is unknown; and (5) we did not analyze brain monoamines after testing to compare the residual effects of the drugs after 21 days of maze learning. For these reasons, further comparisons among the drugs will require future investigation. The reason these additional experiments were not performed was that it was not known in advance what behavioral effects would be found. Now that differences have been shown, more detailed investigations are warranted. Nevertheless, this is the first experiment to compare these drugs on egocentric learning and the data support not only that

they induce deficits in this form of navigation but that drugs affecting dopaminergic systems are more effective than are those affecting serotonergic systems. This raises the question of whether a dopaminergic neurotoxin (such as 6-hydroxydopamine) would also produce egocentric learning impairment.

MA10 affects CWM egocentric learning while sparing MWM allocentric learning (Herring et al., 2008;Herring et al., 2010). Allocentric wayfinding is hippocampally-dependent and relies on distal cues (D'Hooge and De Deyn, 2001). Egocentric learning relies upon proprioceptive self-movement cues, velocity, change of direction, and signposts. Cells in multiple brain regions have been identified that fire in response to an animal's head orientation, including the presubiculum, postsubiculum, anterodorsal thalamus, and lateral mammillary body (Clark et al., 2010). These cells in turn communicate with grid and border cells in the entorhinal cortex and other regions thereby providing a sense of direction (Fuhs and Touretzky, 2006;McNaughton et al., 2006;Rondi-Reig et al., 2006;Sargolini et al., 2006;Solstad et al., 2008;Whishaw et al., 1997;Witter and Moser, 2006).

This form of learning consists of route-based learning and requires paths and nodes (Byrne, 1982) and is observed in all mammalian species (Di and Suarez, 2007). Path integration egocentric learning (Benhamou, 1997;Etienne and Jeffery, 2004;Whishaw, 1998), on the other hand, occurs when an organism returns home directly by vector addition (Shrager et al., 2008;Wolbers et al., 2007). By testing animals in the dark, distal cues were eliminated, and without the possibility to make a vector addition to return home, the CWM is a test of route-based egocentric learning. Hence, the data demonstrate that AMPH and MA induce changes in route-based navigation whereas MDMA and FEN do not (at the doses tested). The present results cannot rule out the possibility that higher doses of MDMA or FEN might not cause route-based deficits.

Egocentric learning can be tested in humans (Shrager et al., 2008;Wolbers et al., 2007) but thus far has not been assessed in drug abusers. Because of this, the relevance of the current findings to the cognitive deficits following chronic MA use remains speculative. In addition, while the data showed no CWM deficits after MDMA treatment under infrared lighting, we previously identified deficits after MDMA under visible red light (Able et al., 2006). The reason for this difference is not known and will benefit from direct comparisons under different lighting conditions.

The present findings should not be interpreted as a direct homology to human cognitive deficits but rather as a putative marker of cognitive compromise that may be useful in future research to further understand how this class of drugs damages learning and memory mechanisms.

Acknowledgments

Research supported by the National Institutes of Health (DA006733).

References

- Able JA, Gudelsky GA, Vorhees CV, Williams MT. 3,4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. *Biol Psychiatry* 2006;59:1219–1226. [PubMed: 16324685]
- Anguiano-Rodriguez PB, Gaytan-Tocaven L, Olvera-Cortes ME. Striatal serotonin depletion facilitates rat egocentric learning via dopamine modulation. *Eur J Pharmacol* 2007;556:91–98. [PubMed: 17126827]

- Bae DD, Brown PL, Kiyatkin EA. Procedure of rectal temperature measurement affects brain, muscle, skin, and body temperatures and modulates the effects of intravenous cocaine. *Brain Res* 2007;1154:61–70. [PubMed: 17466279]
- Baicy K, London ED. Corticolimbic dysregulation and chronic methamphetamine abuse. *Addiction* 2007;102(Suppl 1):5–15. [PubMed: 17493049]
- Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 2004;43:42–51. [PubMed: 15669134]
- Barr AM, Panenka WJ, MacEwan GW, Thornton AE, Lang DJ, Honer WG, Lecomte T. The need for speed: an update on methamphetamine addiction. *J Psychiatry Neurosci* 2006;31:301–313. [PubMed: 16951733]
- Belcher AM, O'Dell SJ, Marshall JF. Impaired object recognition memory following methamphetamine, but not p-chloroamphetamine- or d-amphetamine-induced neurotoxicity. *Neuropsychopharmacology* 2005;30:2026–2034. [PubMed: 15900317]
- Benhamou S. Path integration by swimming rats. *Anim Behav* 1997;54:321–327. [PubMed: 9268463]
- Bisagno V, Ferguson D, Luine VN. Short toxic methamphetamine schedule impairs object recognition task in male rats. *Brain Res* 2002;940:95–101. [PubMed: 12020880]
- Bolla KI, McCann UD, Ricaurte GA. Memory impairment in abstinent MDMA ("Ecstasy") users. *Neurology* 1998;51:1532–1537. [PubMed: 9855498]
- Bowyer JF, Davies DL, Schmued L, Broening HW, Newport GD, Slikker W Jr, Holson RR. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *J Pharmacol Exp Ther* 1994;268:1571–1580. [PubMed: 8138969]
- Bowyer, JF.; Holson, RR. Methamphetamine and amphetamine neurotoxicity. In: Chang, LW.; Dyer, RS., editors. *Handbook of Neurotoxicology*. New York: Marcel Dekker, Inc; 1995. p. 845-870.
- Bowyer JF, Tank AW, Newport GD, Slikker W Jr, Ali SF, Holson RR. The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels and dopamine release in rat striatum. *J Pharmacol Exp Ther* 1992;260:817–824. [PubMed: 1346646]
- Broening HW, Morford LL, Vorhees CV. Interactions of dopamine D₁ and D₂ receptor antagonists with D-methamphetamine-induced hyperthermia and striatal dopamine and serotonin reductions. *Synapse* 2005;56:84–93. [PubMed: 15714503]
- Broening HW, Pu C, Vorhees CV. Methamphetamine selectively damages dopaminergic innervation to the nucleus accumbens core while sparing the shell. *Synapse* 1997;27:153–160. [PubMed: 9266776]
- Byrne, RW. Normality and pathology in cognitive functions. London: Academic Press; 1982. Geographical knowledge and orientation; p. 239-264.
- Cappon GD, Broening HW, Pu C, Morford LL. a-phenyl-N-*tert*-butyl nitron attenuates methamphetamine-induced depletion of striatal dopamine without altering hyperthermia. *Synapse* 1996;24:173–181. [PubMed: 8890459]
- Chang L, Alicata D, Ernst T, Volkow N. Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction* 2007;102(Suppl 1):16–32. [PubMed: 17493050]
- Clark BJ, Bassett JP, Wang SS, Taube JS. Impaired head direction cell representation in the anterodorsal thalamus after lesions of the retrosplenial cortex. *J Neurosci* 2010;30:5289–5302. [PubMed: 20392951]
- Colado MI, Murray TK, Green AR. 5-HT loss in rat brain following 3,4-methylenedioxymethamphetamine (MDMA), p-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocilpine. *Br J Pharmacol* 1993;108:583–589. [PubMed: 7682129]
- Cook D, Kesner RP. Caudate nucleus and memory for egocentric localization. *Behav Neural Biol* 1988;49:332–343. [PubMed: 3408445]
- D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 2001;36:60–90. [PubMed: 11516773]
- Di FA, Suarez SA. Route-based travel and shared routes in sympatric spider and woolly monkeys: cognitive and evolutionary implications. *Anim Cogn* 2007;10:317–329. [PubMed: 17387530]

- Ellison G, Eison MS, Huberman HS, Daniel F. Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. *Science* 1978;201:276–278. [PubMed: 26975]
- EMCDDA. The State of the Drugs Problem in Europe: Annual Report 2007. Luxembourg; 2007. p. 1-100.
- Etienne AS, Jeffery KJ. Path integration in mammals. *Hippocampus* 2004;14:180–192. [PubMed: 15098724]
- Finnegan KT, Karler R. Role for protein synthesis in the neurotoxic effects of methamphetamine in mice and rats. *Brain Res* 1992;591:160–164. [PubMed: 1446227]
- Friedman SD, Castaneda E, Hodge GK. Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav* 1998;61:35–44. [PubMed: 9715805]
- Fuhs MC, Touretzky DS. A spin glass model of path integration in rat medial entorhinal cortex. *J Neurosci* 2006;26:4266–4276. [PubMed: 16624947]
- Gouzoulis-Mayfrank E, Tuchtenhagen F, Pelz S, Becker S, Kunert H-J, Fimm B, Sass H. Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). *J Neurol Neurosurg Psychiatr* 2000;68:719–725. [PubMed: 10811694]
- Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). *Pharmacol Rev* 2003;55:463–508. [PubMed: 12869661]
- Halpern JH, Pope HG Jr, Sherwood AR, Barry S, Hudson JI, Yurgelun-Todd D. Residual neuropsychological effects of illicit 3,4-methylenedioxymethamphetamine (MDMA) in individuals with minimal exposure to other drugs. *Drug Alcohol Depend* 2004;75:135–147. [PubMed: 15276218]
- Han DD, Gu HH. Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol* 2006;6:6. [PubMed: 16515684]
- Harvey J, McMaster S. Fenfluramine: Evidence for a neurotoxic action on midbrain and a long-term depletion of serotonin. *Psychopharmacol Commun* 1975;1:217–228. [PubMed: 1223999]
- Haughey HM, Fleckenstein AE, Hanson GR. Differential regional effects of methamphetamine on the activities of tryptophan and tyrosine hydroxylase. *J Neurochem* 1999;72:661–668. [PubMed: 9930738]
- Haughey HM, Fleckenstein AE, Metzger RR, Hanson GR. The effects of methamphetamine on serotonin transporter activity: Role of dopamine and hyperthermia. *J Neurochem* 2000;75:1608–1617. [PubMed: 10987842]
- He J, Yang Y, Yu Y, Li X, Li XM. The effects of chronic administration of quetiapine on the methamphetamine-induced recognition memory impairment and dopaminergic terminal deficit in rats. *Behav Brain Res* 2006;172:39–45. [PubMed: 16712969]
- Herring, NR.; Gudelsky, GA.; Vorhees, CV.; Williams, MT. (+)- Methamphetamine-induced monoamine reductions and impaired egocentric learning in adrenalectomized rats is independent of hyperthermia. 2010.
- Herring NR, Schaefer TL, Gudelsky GA, Vorhees CV, Williams MT. Effect of (+)-methamphetamine on path integration learning, novel object recognition, and neurotoxicity in rats. *Psychopharmacology (Berl)* 2008;199:637–650. [PubMed: 18509623]
- Hotchkiss AJ, Gibb JW. The long term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J Pharmacol Exp Ther* 1980;214:257–262. [PubMed: 6104722]
- Jacobsen LK, Mencil WE, Pugh KR, Skudlarski P, Krystal JH. Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects. *Psychopharmacology (Berl)* 2004;173:383–390. [PubMed: 14647960]
- Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. College students and adults ages 19–45. Vol. II. Bethesda, MD: National Institute on Drug Abuse, NIH Publication Number 08-6418B; 2008. Monitoring the Future national survey results on drug use, 1975–2007.

- Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. Secondary school students. Vol. I. Bethesda, MD: National Institute on Drug Abuse, NIH Publication Number 09-7402; 2009. Monitoring the Future national survey results on drug use, 1975–2008.
- Kalechstein AD, De La GR, Mahoney JJ III, Fantegrossi WE, Newton TF. MDMA use and neurocognition: a meta-analytic review. *Psychopharmacology (Berl)* 2007;189:531–537. [PubMed: 17082969]
- Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 1997;53:983–997. [PubMed: 9333350]
- Kleven MS, Schuster C, Seiden LS. Effect of depletion of brain serotonin by repeated fenfluramine on neurochemical and anorectic effects of acute fenfluramine. *J Pharmacol Exp Ther* 1988;246:822–828. [PubMed: 2458447]
- Kokoshka JM, Fleckenstein AE, Wilkins DG, Hanson GR. Age-dependent differential responses of monoaminergic systems to high doses of methamphetamine. *J Neurochem* 2000;75:2095–2102. [PubMed: 11032899]
- Kokoshka JM, Metzger RR, Wilkins DG, Gibb JW, Hanson GR, Fleckenstein AE. Methamphetamine treatment rapidly inhibits serotonin, but not glutamate, transporters in rat brain. *Brain Res* 1998;799:78–83. [PubMed: 9666084]
- Krasnova IN, Ladenheim B, Jayanthi S, Oyler J, Moran TH, Huestis MA, Cadet JL. Amphetamine-induced toxicity in dopamine terminals in CD-1 and C57BL/6J mice: complex roles for oxygen-based species and temperature regulation. *Neuroscience* 2001;107:265–274. [PubMed: 11731100]
- London ED, Simon SL, Berman SM, Mandelkern MA, Lichtman AM, Braman J, Shinn AK, Miotto K, Learn J, Dong Y, Matochik JA, Kurian V, Newton T, Woods R, Rawson R, Ling W. Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine users. *Archiv Gen Psychiat* 2004;61:73–84.
- Malberg JE, Seiden LS. Administration of fenfluramine at different ambient temperatures produces different core temperature and 5-HT neurotoxicity profiles. *Brain Res* 1997;765:101–107. [PubMed: 9310399]
- McCann UD, Mertl M, Eligulashvili V, Ricaurte GA. Cognitive performance in ($\pm$) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. *Psychopharmacology* 1999;143:417–425. [PubMed: 10367560]
- McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after (+/-)-3,4-methylenedoxymethamphetamine (MDMA); "Ecstasy": A controlled study in humans. *Neuropsychopharmacology* 1994;10:129–138. [PubMed: 7517677]
- McNaughton BL, Battaglia FP, Jensen O, Moser EI, Moser MB. Path integration and the neural basis of the 'cognitive map'. *Nat Rev Neurosci* 2006;7:663–678. [PubMed: 16858394]
- Meredith CW, Jaffe C, ng-Lee K, Saxon AJ. Implications of chronic methamphetamine use: a literature review. *Harv Rev Psychiatry* 2005;13:141–154. [PubMed: 16020027]
- Metzger RR, Haughey HM, Wilkins DG, Gibb JW, Hanson GR, Fleckenstein AE. Methamphetamine-induced rapid decrease in dopamine transporter function: role of dopamine and hyperthermia. *J Pharmacol Exp Ther* 2000;295:1077–1085. [PubMed: 11082443]
- Monterosso JR, Aron AR, Cordova X, Xu J, London ED. Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug Alcohol Depend* 2005;79:273–277. [PubMed: 15967595]
- Nordahl TE, Salo R, Leamon M. Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: a review. *J Neuropsychiatry Clin Neurosci* 2003;15:317–325. [PubMed: 12928507]
- O'Callaghan, JP.; Miller, DB. Neurotoxic effects of substituted amphetamines in rats and mice: Challenges to the current dogma. In: Massaro, EJ., editor. *Handbook of Neurotoxicity*. Totowa, NJ: Humana Press; 2002. p. 269-301.
- Peat MA, Warren PF, Bakhit C, Gibb JW. The acute effects of methamphetamine, amphetamine and p-chloroamphetamine on the cortical serotonergic system of the rat brain: evidence for differences in the effects of methamphetamine and amphetamine. *Eur J Pharmacol* 1985;116:11–16. [PubMed: 2414113]

- Potegal M. The caudate nucleus egocentric localization system. *Acta Neurobiol Exp (Wars)* 1972;32:479–494. [PubMed: 5075635]
- Pu C, Broening HW, Vorhees CV. Effect of methamphetamine on glutamate-positive neurons in the adult and developing rat somatosensory cortex. *Synapse* 1996;23:328–334. [PubMed: 8855518]
- Pu C, Fisher JE, Cappon GD, Vorhees CV. The effects of amfonelic acid, a dopamine uptake inhibitor, on methamphetamine-induced dopaminergic terminal degeneration and astrocytic response in rat striatum. *Brain Res* 1994;649:217–224. [PubMed: 7953636]
- Pu, C.; Vorhees, CV. Methamphetamine-induced depletion of glutamate-positive neurons in the somatosensory cortex of the rat. 1994. p. 1044
- Pu C, Vorhees CV. Protective effects of MK-801 on methamphetamine-induced depletion of dopaminergic and serotonergic terminals and striatal astrocytic response: An immunohistochemical study. *Synapse* 1995;19:97–104. [PubMed: 7725247]
- Ricaurte GA, Schuster CR, Seiden LS. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Res* 1980;193:153–163. [PubMed: 7378814]
- Riddle EL, Topham MK, Haycock JW, Hanson GR, Fleckenstein AE. Differential trafficking of vesicular monoamine transporter-2 by methamphetamine and cocaine. *Eur J Pharmacol* 2002;449:71–74. [PubMed: 12163108]
- Rondi-Reig L, Petit GH, Tobin C, Tonegawa S, Mariani J, Berthoz A. Impaired sequential egocentric and allocentric memories in forebrain-specific-NMDA receptor knock-out mice during a new task dissociating strategies of navigation. *J Neurosci* 2006;26:4071–4081. [PubMed: 16611824]
- Rothman RB, Ayestas MA, Dersch CM, Baumann MH. Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates: Implications for primary pulmonary hypertension. *Circulation* 1999;100:869–875. [PubMed: 10458725]
- Salo R, Nordahl TE, Moore C, Waters C, Natsuaki Y, Galloway GP, Kile S, Sullivan EV. A dissociation in attentional control: evidence from methamphetamine dependence. *Biol Psychiatry* 2005;57:310–313. [PubMed: 15691533]
- Salo R, Nordahl TE, Natsuaki Y, Leamon MH, Galloway GP, Waters C, Moore CD, Buonocore MH. Attentional control and brain metabolite levels in methamphetamine abusers. *Biol Psychiatry* 2007;61:1272–1280. [PubMed: 17097074]
- Salo R, Nordahl TE, Possin K, Leamon M, Gibson DR, Galloway GP, Flynn NM, Henik A, Pfefferbaum A, Sullivan EV. Preliminary evidence of reduced cognitive inhibition in methamphetamine-dependent individuals. *Psychiatry Res* 2002;111:65–74. [PubMed: 12140121]
- Sargolini F, Fyhn M, Hafting T, McNaughton BL, Witter MP, Moser MB, Moser EI. Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science* 2006;312:758–762. [PubMed: 16675704]
- Scheffel U, Steinert C, Kim SE, Ehlers MD, Boja JW, Kuhar MJ. Effect of dopaminergic drugs on the in vivo binding of [3H]WIN 35,428 to central dopamine transporters. *Synapse* 1996;23:61–69. [PubMed: 8723710]
- Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D. Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. *J Neurosci* 2001;21:5916–5924. [PubMed: 11487614]
- Schroder N, O'Dell SJ, Marshall JF. Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. *Synapse* 2003;49:89–96. [PubMed: 12740864]
- Shoblock JR, Maisonneuve IM, Glick SD. Differences between d-methamphetamine and d-amphetamine in rats: working memory, tolerance, and extinction. *Psychopharmacology (Berl)* 2003a;170:150–156. [PubMed: 12774190]
- Shoblock JR, Sullivan EB, Maisonneuve IM, Glick SD. Neurochemical and behavioral differences between d-methamphetamine and d-amphetamine in rats. *Psychopharmacology (Berl)* 2003b;165:359–369. [PubMed: 12491026]
- Shrager Y, Kirwan CB, Squire LR. Neural basis of the cognitive map: path integration does not require hippocampus or entorhinal cortex. *Proc Natl Acad Sci U S A* 2008;105:12034–12038. [PubMed: 18687893]

- Skelton MR, Able JA, Grace CE, Herring NR, Schaefer TL, Gudelsky GA, Vorhees CV, Williams MT. (+/-)-3,4- Methylendioxyamphetamine treatment in adult rats impairs path integration learning: A comparison of single vs once per week treatment for 5 weeks. *Neuropharmacology* 2008;55:1121–1130. [PubMed: 18674550]
- Skelton MR, Blankenmeyer TL, Gudelsky GA, Brown-Stritthold CA, Vorhees CV, Williams MT. Metyrapone attenuates the sequential learning deficits but not monoamine depletions following d,l-fenfluramine administration to adult rats. *Synapse* 2004;54:214–222. [PubMed: 15484208]
- Solstad T, Boccara CN, Kropff E, Moser MB, Moser EI. Representation of geometric borders in the entorhinal cortex. *Science* 2008;322:1865–1868. [PubMed: 19095945]
- Sprague JE, Preston AS, Leifheit M, Woodside B. Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol Behav* 2003;79:281–287. [PubMed: 12834800]
- Sulzer D, Maidment NT, Rayport S. Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. *J Neurochem* 1993;60:527–535. [PubMed: 8419534]
- Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 2005;75:406–433. [PubMed: 15955613]
- Thompson PM, Hayashi KM, Simon SL, Geaga JA, Hong MS, Sui Y, Lee JY, Toga AW, Ling W, London ED. Structural abnormalities in the brains of human subjects who use methamphetamine. *J Neurosci* 2004;24:6028–6036. [PubMed: 15229250]
- Ugarte YV, Rau KS, Riddle EL, Hanson GR, Fleckenstein AE. Methamphetamine rapidly decreases mouse vesicular dopamine uptake: role of hyperthermia and dopamine D2 receptors. *Eur J Pharmacol* 2003;472:165–171. [PubMed: 12871750]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J Neurosci* 2001a;21:9414–9418. [PubMed: 11717374]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry* 2001b;158:377–382. [PubMed: 11229977]
- Vorhees CV, Herring NR, Schaefer TL, Grace CE, Skelton MR, Johnson HL, Williams MT. Effects of neonatal (+)-methamphetamine on path integration and spatial learning in rats: effects of dose and rearing conditions. *Int J Dev Neurosci* 2008;26:599–610. [PubMed: 18502078]
- Wagner GC, Ricaurte GA, Johanson CE, Schuster CR, Seiden LS. Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. *Neurology* 1980;30:547–550. [PubMed: 6768005]
- Wallace TL, Gudelsky GA, Vorhees CV. Alterations in diurnal and nocturnal locomotor activity in rats treated with a monoamine-depleting regimen of methamphetamine or 3,4-methylendioxyamphetamine. *Psychopharmacology* 2001;153:321–326. [PubMed: 11271404]
- Whishaw IQ. Place learning in hippocampal rats and the path integration hypothesis. *Neurosci Biobehav Res* 1998;22:209–220.
- Whishaw IQ, McKenna JE, Maaswinkel H. Hippocampal lesions and path integration. *Curr Opin Neurobiol* 1997;7:228–234. [PubMed: 9142750]
- Williams MT, Morford LL, McCrear AE, Wood SL, Vorhees CV. Administration of d,l-fenfluramine produces learning deficits in the Cincinnati water maze but not the Morris water maze: Relationship to adrenal cortical output. *Neurotoxicol Teratol* 2002;24:1–14. [PubMed: 11836066]
- Witter MP, Moser EI. Spatial representation and the architecture of the entorhinal cortex. *Trends Neurosci* 2006;29:671–678. [PubMed: 17069897]
- Wolbers T, Wiener JM, Mallot HA, Buchel C. Differential recruitment of the hippocampus, medial prefrontal cortex, and the human motion complex during path integration in humans. *J Neurosci* 2007;27:9408–9416. [PubMed: 17728454]

- Yoshida, T. Use and misuse of amphetamines: An international overview. In: Klee, H., editor. Amphetamine Misuse: International Perspectives on Current Trends. The Netherlands: Harwood Academic Publishers; 1997. p. 1-16.
- Zaczek R, Battaglia G, Culp S, Appel N, Contrera J, De Souza E. Effects of repeated fenfluramine administration on indices of monoamine function in rat brain: pharmacokinetic, dose response, regional specificity and time course data. *J Pharmacol Exp Ther* 1990;253:104–112. [PubMed: 2329498]

Figure 1.

Body temperatures: Mean $\pm$ SEM body temperature recordings from subcutaneously implanted temperature transponders in rats treated with Saline, MA, AMPH, MDMA, or FEN every 2 h. The arrows denote times when drug injections were delivered. Average ambient temperature during treatment was 23.1°C. Asterisks on brackets indicate significant differences for the designated drug groups throughout the designated range. Group sizes: Saline = 21, MA10 = 21, MA12.5 = 18, AMPH = 16, MDMA = 21, FEN = 21. * $P < 0.05$ or less.

Figure 2.

Learning curves for the CWM: Data are mean $\pm$ SEM total errors per day. **A** = total errors in the Saline and AMPH groups; **B** = total errors in the Saline, MA10, and MA12.5 groups; **C** = total errors in the Saline, MDMA, and FEN groups. Significant differences among the groups are shown in Fig. 3. Group sizes are as in Fig. 1.

Figure 3. CWM total errors: Data are mean $\pm$ SEM total errors per day plotted for each drug treatment group averaged across days of testing. Group sizes are as listed in Fig. 1. * $P < 0.05$ vs Saline; *** $P < 0.001$ vs. Saline.

Table 1

Body weight (g) of rats immediately prior to treatment and at 72 h, 2 and 4 weeks post-treatment

	Pre-treatment	Post-treatment		
		72 h	2 wks	5 wks
Saline	440.1 ± 14.0	442.1 ± 13.8	475.7 ± 15.8	516.0 ± 13.6
MA10	442.4 ± 12.2	420.9 ± 11.1 *	460.8 ± 10.5	516.8 ± 11.8
MA12.5	446.1 ± 11.5	409.3 ± 9.5 ***	447.0 ± 9.0	516.4 ± 11.8
AMPH	443.4 ± 12.5	398.7 ± 15.5 ***	443.1 ± 12.7	514.6 ± 12.1
FEN	445.7 ± 15.3	418.3 ± 13.4 **	471.4 ± 12.8	526.3 ± 12.8
MDMA	438.0 ± 12.4	425.5 ± 10.5 *	463.6 ± 8.8	515.6 ± 10.9

*
P<0.05 ;**
P<0.01 ;***
P<0.001 vs Saline

Table 2

Intercorrelation coefficients among dependent variables recorded in the CWM with correction for animals not finding the goal (A) and without correction for animals that not finding the goal (B)

A	Corrected				
	Tot err	T+R	T	S	R
Tot err	1.0	0.982	0.981	0.986	0.955
T+R		1.0	0.999	0.997	0.984
T-only			1.0	0.997	0.984
S-only				1.0	0.979
R-only					1.0
Lat					1.0
B	Uncorrected				
	Tot err	T+R	T	S	R
Tot err	1.0	0.991	0.985	0.981	0.857
T+R		1.0	0.991	0.946	0.874
T			1.0	0.944	0.802
S				1.0	0.806
R					1.0
Lat					1.0

Cincinnati water maze total errors: Mean ($\pm$ SEM) errors/day (N) across 21 days of testing (2 trials/day) for subsets of animals that reached a core temperature of 40.2°C and were cooled versus those that did not reach 40.2°C and were not cooled

Table 3

	Saline	MA10	MA12.5	AMPH	FEN	MDMA
Cooled	----	32.8 $\pm$ 4.0 (10)	33.5 $\pm$ 16.6 (11)	42.3 $\pm$ 5.2 (8)	----	25.6 $\pm$ 3.1 (7)
Not Cooled	24.9 $\pm$ 1.9 (21)	30.6 $\pm$ 4.0 (10)	31.6 $\pm$ 16.6 (8)	36.6 $\pm$ 5.6 (8)	27.4 $\pm$ 1.7 (21)	29.3 $\pm$ 2.2 (14)