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Reversal-Specific Learning Impairments After a Binge Regimen of Methamphetamine in Rats: Possible Involvement of Striatal Dopamine

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A growing body of evidence indicates that protracted use of methamphetamine (mAMPH) causes long-term impairments in cognitive function in humans. Aside from the widely reported problems with attention, mAMPH users exhibit learning and memory deficits, particularly on tasks requiring response control. Although binge mAMPH administration to animals results in cognitive deficits, few studies have attempted to test behavioral flexibility in animals after mAMPH exposure. The aim of this study was to evaluate whether mAMPH would produce impairments in two tasks assessing flexible responding in rats: a touchscreen-based discrimination-reversal learning task and an attentional set shift task (ASST) based on a hallmark test of executive function in humans, the Wisconsin Card Sort. We treated male Long-Evans rats with a regimen of four injections of 2 mg/kg mAMPH (or vehicle) within a single day, a dosing regimen shown earlier to produce object recognition impairments. We then tested them on (1) reversal learning after pretreatment discrimination learning or (2) the ASST. Early reversal learning accuracy was impaired in mAMPH-treated rats. MAMPH pretreatment also selectively impaired reversal performance during ASST testing, leaving set-shifting performance intact. Postmortem analysis of [^{125}I]RTI-55 binding revealed small (10–20%) but significant reductions in striatal dopamine transporters produced by this mAMPH regimen. Together, these results lend new information to the growing field documenting impaired cognition after mAMPH exposure, and constitute a rat model of the widely reported decision-making deficits resulting from mAMPH abuse seen in humans.
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INTRODUCTION

Methamphetamine (mAMPH) is a highly addictive psychostimulant drug that can result in impaired cognition in humans (Ornstein *et al*, 2000; Simon *et al*, 2000; Bechara *et al*, 2001; Volkow *et al*, 2001c). Research on human users suggests that the cognitive deficits extend beyond learning and memory, into the realm of inhibitory control and executive function (Rogers *et al*, 1999; Ornstein *et al*, 2000; Simon *et al*, 2000; Bechara *et al*, 2001; Kalechstein *et al*, 2003; Nordahl *et al*, 2003; McCann *et al*, 2008). The use of animal experimental models can help elucidate the neurobiological mechanisms by which abuse of this drug brings about such cognitive sequelae. Many researchers have used animal models of mAMPH exposure that incorporate short, moderate-to-high dose schedules of mAMPH administration, intended to mimic human abusers' bingeing patterns of mAMPH use. In addition to uncovering brain mechanisms, studies of drug-exposed animals help address the complicated question of whether cognitive impairments are a direct consequence of the drug, secondary to other conditions arising from the drug, or representative of preexisting vulnerabilities in the population of drug users—vulnerabilities that predispose them to becoming addicts (Verdejo-Garcia *et al*, 2008). Administering acute, binge doses of mAMPH to rats produces impairments in several domains of learning and memory (Bisagno *et al*, 2002; Schroder *et al*, 2003; Belcher *et al*, 2005; He *et al*, 2006; Herring *et al*, 2008a) and often without evidence of neurotoxicity (Belcher *et al*, 2006; Nagai *et al*, 2007).

At sufficiently high doses, binge mAMPH exposure causes dose-dependent damage to the dopamine (DA) system, injury that has been reported in both nonhuman primates and rodents (Ricaurte *et al*, 1982; Gibb *et al*, 1987; O'Dell

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et al, 1993; Villemagne *et al*, 1998), with the ventral region of caudate-putamen (CP) being particularly vulnerable to mAMPH's neurotoxic effects (Eisch *et al*, 1992). Converging evidence suggests that human mAMPH abusers also have reductions in brain DA indices (Eisch *et al*, 1992; Wilson *et al*, 1996; Volkow *et al*, 2001b; Volkow *et al*, 2001a; Volkow *et al*, 2001c).

Compulsive drug-seeking behavior bears a striking resemblance to the disinhibited behavior after damage to the frontal cortex and is thought to be due, in part, to the many plastic responses that occur in the brain after repeated psychostimulant use (Everitt and Robbins, 2005; Dalley *et al*, 2008; Everitt *et al*, 2008). In fact, changes in the striatum may be at the root of such compulsive behavior (Everitt and Wolf, 2002), with measures of enhanced DA activity in dorsal striatum associated with higher self-reported levels of craving and strength of psychostimulant habit in humans (Volkow *et al*, 2006) and increasing 'habitization' of behavior in rats (Takahashi *et al*, 2007). In addition, lesions of the medial or ventral striatum produce perseverative, compulsive responding on reversal learning in monkeys (Clarke *et al*, 2008) and strategy set shifting in rats (Block *et al*, 2007), both assays for different forms of behavioral flexibility. These effects are similar to those well documented after lesions to the ventromedial prefrontal cortex, namely the orbitofrontal cortex (OFC; Schoenbaum and Shaham, 2008).

Given the foregoing, there appears to be an association between the neurobiological consequences of heavy psychostimulant use and compromised inhibitory control mechanisms. To date, animal studies of mAMPH effects on cognition have focused on testing for impairments using traditional learning and memory paradigms. Surprisingly, with few exceptions (Dalley *et al*, 2007; Daberkow *et al*, 2008) there has been relatively little development in an animal behavioral model investigating the long-term effects of mAMPH on behavioral flexibility. To our knowledge, there have been no investigations of discrimination reversal learning and attentional set shifting after binge-type administrations of mAMPH. The aim of this study was to investigate the effects of binge mAMPH exposure on two hallmark measures of flexible behavior in rats: visual discrimination reversal learning and attentional set shifting. We chose a treatment regimen of four injections of 2 mg/kg mAMPH because this regimen results in (1) acute hyperthermia during treatment, (2) a posttreatment object recognition impairment, and (3) significant but moderate ($\sim$20%) depletions of DA transporter in ventral CP while sparing the serotonin transporter in the areas examined (hippocampus, perirhinal cortex) (Belcher *et al*, 2008). This dose is also well tolerated by the exposed animals. As widespread neurotoxicity appears not to result from treatment with this moderate dose, we focused our quantification of DAT to the striatum, the area in the brain most sensitive to the DA-depleting effects of mAMPH (Eisch *et al*, 1992).

MATERIALS AND METHODS

Subjects

Thirty-seven adult male Long-Evans hooded rats (275–300 g at start of experiment) were obtained from Charles River Laboratories (Raleigh, NC) and individually housed, with water *ad libitum*, under a standard 12 h-light/12 h-dark cycle (lights on 0700–1900 h) at a temperature of 22°C. Rats were food restricted to 85% free-feeding body weight for behavioral testing (see Experiments 1 and 2). Protocols for this research were approved by the Institutional Animal Care and Use Committees of the University of California, Irvine and California State University, Los Angeles. Acquisition, maintenance, handling, procedures, and care of the animals were in accord with the NIH Guide for the Care and Use of Laboratory Animals.

Drug Treatments

On the day of mAMPH injections, rats were kept in large, clear Plexiglas cages measuring 40 cm (length) $\times$ 40 cm (width) $\times$ 38 cm (height) in groups of no more than seven animals each. Ambient room temperature was kept at 23 ± 1.5°C. Rats were given injections of d-mAMPH (Sigma, St Louis, MO; 2 mg free base/kg, sc) or physiological saline solution (SAL; 1 ml/kg, sc) at 2-h intervals for a total of four injections. This dose was chosen to minimize motor impairments and to circumvent any stress response produced by an interaction with food restriction, given the known deleterious effects of stress on behavioral flexibility (Holmes and Wellman, 2008). Animal body temperatures were monitored 60 min after each injection: by rectal probe (Experiment 1) or by temperature transponders (IPTT-300; BioMedic Data Systems, Seaford, DE) implanted subcutaneously along the dorsum between the animals' scapula the day before drug treatments (Experiment 2); Table 1.

Table 1 Body Temperatures of Rats Used in Experiment 1 (Visual Discrimination Reversal) and Experiment 2 (Attentional Set Shift Task)

	Time 0	1 h after first injection	1 h after second injection	1 h after third injection	1 h after fourth injection
Experiment 1 saline		38.5 ± 0.1	37.6 ± 0.3	36.6 ± 1.4	37.0 ± 1.8
Experiment 1 mAMPH		38.9 ± 0.3	38.5 ± 0.4	38.8 ± 0.2	39.5 ± 0.4
Experiment 2 saline	36.6 ± 0.6	37.8 ± 0.1	36.6 ± 0.2	36.5 ± 0.3	36.7 ± 0.2
Experiment 2 mAMPH	36.9 ± 0.4	39.5 ± 0.3	38.8 ± 0.5	39.2 ± 0.4	39.0 ± 0.5

Temperatures (°C) were recorded 1 h after each injection as well as before the injection regimen ('Time 0', for Experiment 2 only).

Experiment 1. Visual Discrimination and Reversal Learning

Rats used in this experiment were acclimated to the testing room for 15 min and fed immediately on returning to the homecage. All behavioral testing took place between 0800 h and 1600 h 5–6 days per week. As outlined in the steps below, rats were shaped, pretrained to nosepoke a touch-sensitive screen, and trained on a discrimination problem (eg, stimulus pair) all before treatment with drug. They were then treated with mAMPH or SAL, given a posttreatment 'retention test' and administered a reversal of reward contingency.

Apparatus. Operant chambers (#80004, Lafayette Instrument Co., Lafayette, IN), measuring 35.6 cm (length) × 27.9 cm (width) × 33.7 cm (height) were each housed within a sound- and light-attenuating cubicle (#83018DDP Lafayette Instrument Co., Lafayette, IN). Each operant chamber was outfitted with a touch-sensitive, 12″ LCD flat screen (EloTouch, Menlo Park, CA). The chamber floor was covered with a clear Plexiglas sheet to facilitate mobility. The touchscreen and a single houselight were located at one end of the chamber; a tone generator, a pellet receptacle and a pellet dispenser, at the other end. The pellet dispenser delivered 45 mg dustless sucrose pellets (BioServ, Frenchtown, NJ). Stimulus presentation, reward delivery and contingencies were controlled by custom-designed software developed for use in nonhuman primate experiments (Ryklin Software, Inc). The equiluminant stimuli were the same as those reported in an earlier study (Izquierdo *et al*, 2006b).

Behavioral testing.

Handling and accommodation to food rewards: One week on arrival to the vivarium, each rat was handled for a minimum of 10 min once per day for 5 days before behavioral testing. During this time, animals were given a small amount of 45 mg sucrose pellets (~20 pellets) in their homecage after each day of handling to accustom them to the food rewards.

Food restriction: Food restriction to no more than 85% free-feeding body weight began during the handling period and when rats weighed a minimum of 275 g (between 8 and 10 weeks of age). Rats were weighed three times per week to ensure a healthy body weight throughout testing.

Acclimation, shaping, and pretraining: During acclimation, rats were required to eat pellets out of the pellet tray before exposure to any stimuli on the touchscreen. Shaping then involved the concurrent disappearance of a stimulus presented on the touchscreen and a 'reward event': illumination of a houselight in the sound-attenuating cubicle, illumination of the pellet tray light, onset of 2-s auditory tone, and provision of a 45-mg sucrose pellet. At any point during shaping, rats could be rewarded for a 'nosepoke' on the touchscreen by this 'reward event.' Criterion for shaping occurred when rats ate 60 sucrose pellets within 30 min.

Pretraining involved three cumulative stages: (1) touch—rats must touch the stimulus on the touchscreen by nosepoking the stimulus; (2) initiate—rats must initiate the onset of the next trial by nosepoking the pellet receptacle door; (3) punish—rats get 'punished' by a (house)light-out, the absence of the pellet receptacle light, and the absence of the auditory tone that usually signals reward. Instead, the trial is 'timed out,' rendering rats unable to initiate the next trial for 5 s. Criterion for each phase of pretraining was 60 completed trials (touches) and no pellets remaining in the pellet receptacle in 30 min.

Visual discrimination learning: Rats were presented with two two-dimensional, equiluminant white stimuli on a black background and trained according to predetermined reinforcement contingencies: Stimulus A resulted in a food reward (A+), whereas nosepoking the other stimulus, B, resulted in a 5 s timed-out punishment (B−). Designation of the rewarded stimulus was counterbalanced across treatment groups. The custom software enabled stimuli to be presented on the screen indefinitely until the animal nosepoked one of the stimuli. Only small preprogrammed 'response windows' overlying the stimuli were sensitive to nosepoking: nosepoking outside of the response window was undetected; nosepoking within it was either correct or incorrect, depending on reward contingency. Left/right presentation of the S+ was pseudorandom, according to a Gellerman schedule generated by Ryklin Software Inc. There were 60 total trials per session (and one session per day) with a 10-s ITI. For this learning phase, rats were required to reach a criterion of 85% correct out of 60 trials across each of 2 consecutive days. Performance was assessed according to three measures: session percent correct, session perseveration index (P.I., measured by dividing the number of consecutive errors in a row before switching response by the total number of errors within a session), and the number of sessions to reach performance criterion of 85% correct across two sessions.

All rats were given a 'reminder' session the day before treatment to account for differences in the recency of exposure to the reward contingencies in discrimination learning.

Posttreatment retention and reversal learning: After treatment with mAMPH, rats were given 3–5 days of rest (eg, no behavioral testing, in homecage), followed by a test for retention of the discrimination problem. Rats were then required to respond to a reversal in reward contingency: nosepoking the previously incorrect stimulus was now rewarded by provision of a sucrose pellet. Thus, testing on reversal learning began 5–8 days post-mAMPH or SAL treatment. As in the earlier phase, criterion was set at a mean score of 85% correct out of 60 trials across two consecutive sessions. Performance was assessed according to the same three measures described above.

Experiment 2. Attentional Set Shift Task

Apparatus. A separate group of male, Long-Evans rats weighing 275–300 g at the time of mAMPH treatments was used. Attentional set shift task (ASST) procedures were conducted according to Birrell and Brown (2000). Rats were trained in a Plexiglas arena that measured 36.8 cm (height) × 45.7 cm (width) × 68.6 cm (length). The box was divided equally into thirds so that each compartment was

22.9 cm long. The front of the apparatus was further divided into two separate sections where the bowls were contained separately, to avoid animals having access to both bowls simultaneously. In addition, access to each compartment (and bowl) at the front of the box could be restricted by an opaque, removable divider. The back third of the apparatus (inter-trial chamber) was separated from the other compartments with a removable divider, and rats were placed in that chamber at the beginning of each trial. Access to the inter-trial chamber was blocked once a trial was in progress. Food rewards (one-half of a Frosted Cheerio, General Mills, Golden Valley, MN) were buried half-way down ceramic bowls (3.8 cm tall having an internal diameter of 8.3 cm). Rats were trained on successive days to make discriminations based on two dimensions: media of varying textures (eg, vermiculite, confetti, gravel), or scents (eg, paprika, thyme or oregano). Scents could be mixed interchangeably with media so that combinations of the two dimensions were possible, but pairs of scents or media were kept constant (eg, cumin was always presented with cinnamon; vermiculite was always presented with gravel).

Behavioral testing.

Food restriction: Rats were given 3–5 days of rest (eg, no behavioral testing, in homecage) after mAMPH or SAL injections before food restriction began, allowing for weight stabilization before food restriction. During food restriction, rats were fed 15 g of food per day, and weight was monitored daily to maintain a target weight of 85% normal weight. To habituate the animals to the feeding bowls, and to familiarize them with digging for rewards, food was given in small ceramic bowls in the home-cage for several days before training. ASST training began on the 14th day of food deprivation. Rats were returned to free feeding once testing was complete, and they were kept on food deprivation for no more than 30 days.

Habituation: After the 14-day food-deprivation (17–19 days after mAMPH or SAL injections), rats were habituated to the arena for 5 min before training. To familiarize the animals with digging for a food reward, animals were presented with two baited unscented bowls filled with home cage bedding. Once the animal had retrieved the food reward, the animal was moved back to the inter-trial chamber and the bowls were re-baited. The divider was then lifted, allowing the animal access to the bowls. All subsequent trials were conducted in this same manner. Once the animal was reliably digging for a food reward, training on the task was begun.

Training: During the training phase, rats were trained on two simple discriminations (SDs), one scent-based (thyme *vs* paprika), and the second media-based (vermiculite *vs* plastic beads). All rats were trained on the same discriminations, in the same order. Criterion completion of training was achieved when the rat made a correct discrimination for six consecutive trials. Most rats completed this training during a single day. Rats that did not learn to dig for food reward within the day were returned to their home-cage and a second attempt at training was made 1–2 days later. A maximum of three attempts were made to train each rat, with overall success in $>90\%$ of the animals.

Testing paradigm: The day after rats had completed training on the two SD training trials, testing began. A trial was initiated by raising the dividers, giving the rat access to the two bowls, only one of which was baited. For each phase, the rat was given four discovery trials, whereby the rat was allowed to dig in both bowls (even though only one was baited) to retrieve the food reward. Errors were recorded during the discovery trials, but did not count toward trials to criterion (Birrell and Brown, 2000). On subsequent trials, if the rat dug in the unbaited (incorrect) bowl, an error was recorded, and the trial was terminated. In a single-test session, rats were given the following discrimination phases to learn: (1) SD, a scent-based discrimination (nutmeg* *vs* cloves); (2) compound discrimination (CD), media (paper squares *vs* shredded paper) dimension is introduced, scent is still rewarded, irrespective of medium (nutmeg*/paper squares and nutmeg*/shredded paper); (3) CD-reversal (CDr), previously unrewarded scent is now rewarded, irrespective of medium (cloves*/paper squares and cloves*/shredded paper); (4) intradimensional shift (ID), animal must still attend to scent and correctly discriminate the rewarded scent, but novel scents (cinnamon* *vs* cumin) and media (foam triangles *vs* straws) are introduced; (5) ID-reversal (IDr), previously unrewarded scent is now rewarded, irrespective of medium (cumin*/foam triangles and cumin*/straws); (6) extradimensional shift (ED), rat is trained to attend to medium cues ($\frac{1}{4}$ foam shells* *vs* crushed foam) and ignore scent cues (celery seed *vs* sumac); (7) ED-reversal (EDr), previously unrewarded medium is now rewarded, irrespective of scent (crushed foam*/celery seed and crushed foam*/sumac). During each phase, the rats were tested until they had achieved a criterion of six consecutive correct choices. Two measures were used to quantify performance in each phase: (1) trials-to-criterion (the number of trials taken to reach criterion), and (2) the number of errors made in reaching criterion. The order of the discriminations and the exemplar pairings were always the same, but the pairs of exemplars were counterbalanced between groups. Typically, rats completed all phases of testing within a single day.

Experiment 3. [^{125}I]RTI-55 Binding to DA Transporters

A separate group of male, Long-Evans rats weighing 275–300 g at the time of treatment was given binge mAMPH (4×2 mg/kg, s.c., $N=6$) or saline (0.9% sterile saline, s.c., $N=3$) injections as described above and was killed 1 week later. The reason for the use of a separate group of rats was to afford an accurate estimate of DAT binding at a time point that corresponded with commencement of testing in Experiment 1. Rats were anesthetized with an overdose of sodium pentobarbital (250 mg/kg, i.p.), decapitated, and their brains removed and frozen at -20°C by immersion in isopentane. A measure of 20 μm-thick coronal sections were cut on a cryostat at the level of the anterior striatum (AP coordinates $+1.7$ mm to $+0.8$ mm, according to Paxinos and Watson, 2003), thaw-mounted on Vectabond-treated glass slides and stored at -20°C until used for autoradiography. Warmed slides removed from the -20°C freezer were preincubated in a solution of assay buffer (10 mM $NaPO_4$, 120 mM NaCl, 100 mM sucrose) containing 100 nM fluoxetine for 5 min to remove endogenous ligands that

could interfere with subsequent radioligand binding. As [^{125}I]RTI-55 binds with high affinity to both DAT and SERT, 100 nM fluoxetine was included in both the preincubation and incubation media to block radioligand binding to SERT (Boja *et al*, 1992) . After preincubation, the sections were incubated in a solution of assay buffer containing 25 pM [^{125}I]RTI-55 and 100 nM fluoxetine for 2 h. The sections were then rinsed twice for 2 min each at 4°C in assay buffer, then once for 10 s in 4°C distilled water. The rinsed slides were then rapidly dried under a stream of heated air. The dried slides and [^{14}C]-containing autoradiographic standards were apposed to Hyperfilm MP (GE Healthcare) for 48 h before development.

Quantification of [^{125}I]RTI-55 binding was done using an MCID image analyzer (InterFocus Imaging; Cambridge, England). Image densities were converted to [^{125}I]RTI-55 binding levels using a calibration curve based on images of the standard slides packed with each film. Regional densities of RTI binding were obtained by outlining the desired structures on their respective [^{125}I]RTI-55 images. Values obtained represented the average of measurements taken from both hemispheres in a total of four sections per animal. For analysis, the image of striatum was first subdivided into CP and nucleus accumbens septi (NAc). The CP was then subdivided into dorsal (dCP) and ventral (vCP) parts, which were separately quantified for [^{125}I]RTI-55 binding (see Figure 4).

Statistics. Temperature data were analyzed using repeated-measures ANOVA. Visual discrimination reversal learning data (percent correct and P.I.) were analyzed with repeated-measures ANOVA for early (sessions 1–3), middle (sessions 4–6), and late (sessions 7–9) learning phases. Retention of visual discrimination and sessions to criterion were assessed using independent-samples *t*-tests. ASST behavioral data (trials to criteria, errors) and [^{125}I]RTI-55 binding data were assessed using repeated measures ANOVA. *t*-Tests were used to compare performance of mAMPH and SAL groups during each phase of the ASST. Within-group comparisons between performance in the ED and ID phases were performed by paired *t*-tests. In all instances, two-tailed tests were used, with *p*-values equal to and <0.05 considered statistically significant and trends noted at *p*-values less than and equal to 0.10. Data are presented as mean ± SEM values and were analyzed with SPSS.

RESULTS

Experiment 1

Effect of mAMPH on visual discrimination: pretreatment learning and posttreatment retention. Rats learned the visual discrimination problem in an average of 3.2 sessions (± 1.4 SEM). Stimulus-reward assignment (either A + B− or A−B +) did not influence the rate of acquisition, thus data were collapsed for all subsequent analyses.

One day before treatment with mAMPH or SAL, rats were given a 'reminder' session of the discrimination problem. They were then tested for their retention of the initial visual discrimination problem after treatment. As shown in Figure 1, the mAMPH and SAL groups scored similarly on this memory test (percent correct: mAMPH, 89.97 ± 1.26; SAL, 87.98 ± 3.62; $t(10) = 0.59$, $p = 0.57$).

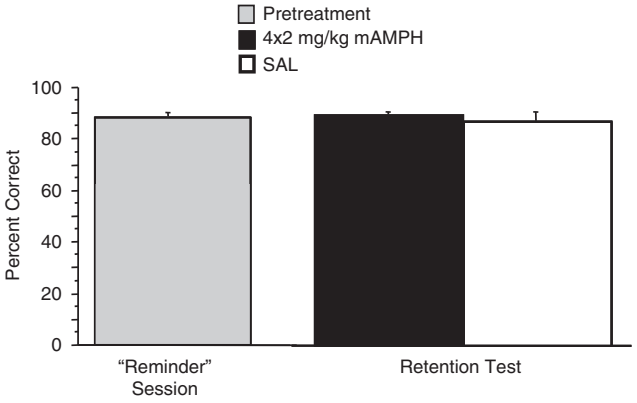

Figure 1 Retention of a pretreatment visual discrimination problem. Rats were given a 'reminder session' where they were exposed to the discrimination problem just learned. Three days after treatment with mAMPH or SAL, they were given a retention test. There were no significant differences between the mAMPH- and SAL-treated groups ($n = 7$ mAMPH, $n = 5$ SAL). Data are means ± SEM.

Effect of mAMPH on reversal learning. Consistent with the known acute effects of this mAMPH dose (4 × 2 mg/kg, s.c.), the mAMPH-treated rats showed a trend toward elevated body temperatures during the treatment regimen (Table 1; $F_{1,9} = 3.41$, $p = 0.06$).

Although there were no significant treatment differences on overall sessions to criterion (additional details below), we explored early reversal learning for two reasons: (1) initial reversal learning taps into greater inhibitory control and constitutes the phase in which the greatest perseveration is seen (Jones and Mishkin, 1972) and (2) dopaminergic manipulations selectively affect early reversal learning using touchscreen-response methodology in rodents (Izquierdo *et al*, 2006b). Thus, repeated-measures ANOVA were used to assess treatment differences on each phase of learning: early (sessions 1–3), middle (sessions 4–6), and late (sessions 7–9) reversal learning. As learning rates differed across animals (eg, two mAMPH-pretreated rats and one SAL-pretreated rat lacked nine full sessions), rats' two-session criterion average of 85% or better was carried forward to complete late phase reversal learning. There was a significant effect of treatment in early reversal performance such that the mAMPH-pretreated group was significantly impaired relative to the control group (percent correct: mAMPH, 29.4 ± 4.2; SAL, 44.0 ± 4.5; $F_{1,10} = 4.6$, $p = 0.05$; data shown in Figure 2). There was no significant treatment × session interaction in early, middle, or late phases of learning, but significant effects of session were observed: all rats showed evidence of improvement with increasing session number (all F-values >2.0, all *p*-values <0.05). Importantly, there were no differences between treatment groups on middle or late reversal learning. There were also no differences in Perseveration Index between treatment groups during early, middle, or late phases. Stimulus-reward assignment (either A + B− or A−B +) did not explain the treatment difference in percent correct scored during early reversal, nor was there a significant

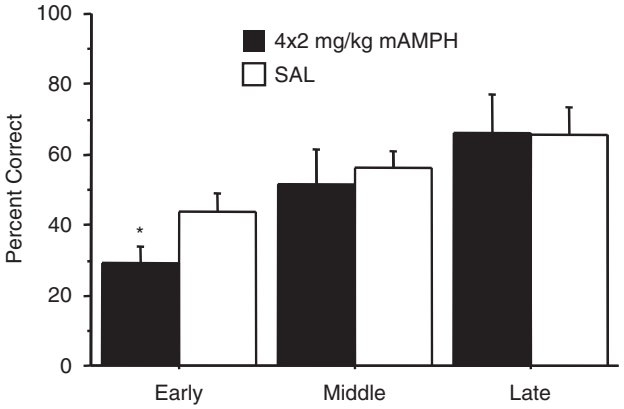

Figure 2 Early discrimination reversal learning differs between mAMPH- and SAL-treated groups. Performance on reversal learning was subdivided into early (sessions 1–3), middle (sessions 4–6), and late (session 7–9) phases. MAMPH-pretreated rats scored lower percent correct on early reversal phase only ($n=7$ mAMPH, $n=5$ SAL). Bars represent group means ± SEM *$p=0.05$ *vs* SAL.

interaction of stimulus-reward assignment by session. As stated earlier, overall rate of learning on reversal of reward contingencies was not different between treatment groups (sessions to criterion: mAMPH, 11.6 ± 2.8; SAL, 12.2 ± 2.4; $t(10)=-0.16$, $p=0.88$).

Experiment 2

ASST performance. The mAMPH-treated rats (4 × 2 mg/kg, s.c.) subsequently tested for ASST performance showed a significant elevation in body temperatures during the treatment regimen (Table 1; $F_{1,8}=10.44$, $p<0.02$).

During training, the rats readily learned to discriminate between food-baited bowls based on either scents or media. Testing was conducted over seven phases, as described in Materials and Methods and Figure 3. Repeated measures ANOVA was used to investigate differences in trials-to-criteria and errors made for the mAMPH and SAL groups. For the trials-to-criterion measure, the effects of treatment (SAL *vs* mAMPH; $F_{1,14}=5.87$, $p=0.03$) and phase ($F_{6,84}=29.37$, $p<0.001$) and the phase × treatment interaction ($F_{6,34}=3.17$, $p=0.007$) were significant. Subsequent tests revealed that the mAMPH group took significantly more trials to reach criteria than did SAL animals for the IDr ($F_{1,14}=20.5$, $p<0.001$) and EDr ($F_{1,14}=4.94$, $p=0.04$) phases and showed a trend toward treatment difference in the CDr phase ($F_{1,14}=3.09$, $p=0.10$). For the errors measure, the effects of treatment ($F_{1,14}=4.51$, $p=0.05$) and phase ($F_{6,84}=22.5$, $p<0.001$) were significant but the interaction was not ($F_{6,84}=1.75$, $p=0.12$).

In contrast to the effects of mAMPH pretreatment on reversals, no evidence of impairment in shifting of attention was found. That is, both mAMPH- and SAL-pretreated groups required more trials to reach criteria, and committed more errors, for the discrimination requiring an extradimensional shift, compared with intradimensional shift (paired sample *t*-tests, *p*-values < 0.025). However, the ED–ID difference scores of the two groups did not differ significantly, suggesting that mAMPH did not influence animals' attentional set-shifting abilities.

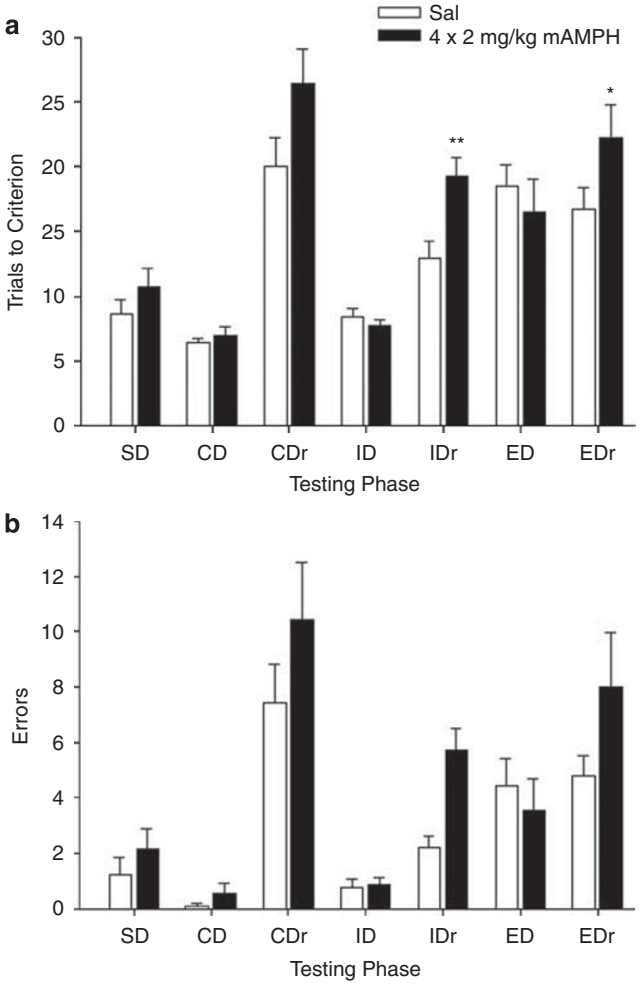

Figure 3 Reversal learning is impaired and set shifting is intact in the ASST task after mAMPH treatment. (a) mean (± SEM) trials to reach criterion (six successive correct trials) for each phase of the attentional set shift paradigm. (b) mean (± SEM) errors made in each phase of the ASST paradigm ($n=9$ mAMPH, $n=7$ SAL). See Materials and Methods for details. *$p\leqslant 0.05$; **$p<0.001$.

Experiment 3

[^{125}I]RTI-55 binding to DA and serotonin transporters. Analysis of [^{125}I]RTI-55 binding revealed that treatment of rats with 4 × 2 mg/kg, s.c. mAMPH regimen produced a significant (~14%) depletion in DAT within CP, whereas NAc DAT was unaffected. Further analysis of the CP revealed that the mAMPH-induced reductions in DAT occurred in both the dorsal and ventral subdivisions, with the greatest effect being evident in the ventral region (with depletions averaging about 18%, relative to controls; Figure 4).

DISCUSSION

To our knowledge, the current report offers the first evidence of binge mAMPH-induced deficits in two forms of discrimination reversal learning, a widely used assay of flexible cognition. These results fit well with findings of human mAMPH-dependent individuals' executive dysfunction, especially when these individuals are confronted with

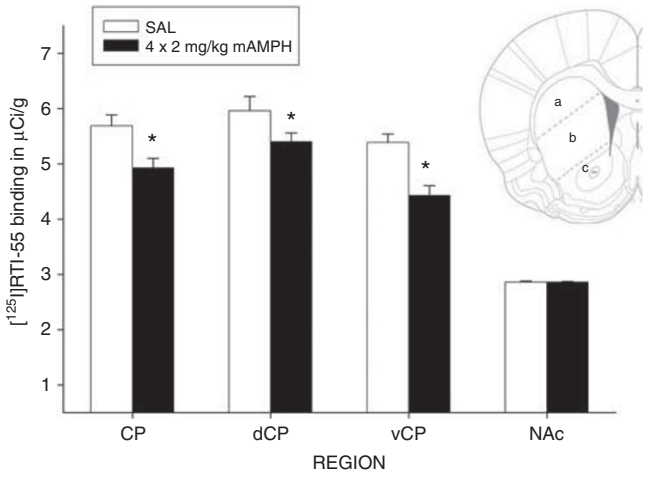

Figure 4 [^{125}I]RTI-55 binding to striatal dopamine transporters (DAT). Mean ± SEM values (in µCi/g) for DAT binding in the striatum of Long-Evans rats exposed to 4 × 2 mg/kg, s.c. methamphetamine (mAMPH; $N = 6$) or saline (SAL; $N = 3$) dosing regimen 1 week earlier. Values were generated by quantitative autoradiography of ligand binding. Initial readings were taken from whole caudate-putamen (CP, areas labeled a and b), followed by readings in the dorsal (dCP, labeled a) and ventral (vCP, labeled b) subdivisions of the CP, and in the nucleus accumbens septi (NAc, labeled c). Representative section = Bregma + 1.44 mm. *mAMPH different from SAL, $p < 0.02$.

changes in contingencies (Rogers *et al*, 1999; Kim *et al*, 2005; Chung *et al*, 2007; Han *et al*, 2008). This inability to update responding in favor of more adaptive choices is reminiscent of the impairments seen after damage to OFC. Altered striatal dopaminergic function and its relationship to OFC function are discussed below.

Reversal-Specific Impairment After 4 × 2 mg/kg mAMPH Treatment

Rats treated with a single-day binge regimen of mAMPH (4 × 2 mg/kg) were impaired on reversal learning as assessed in two tasks: visual discrimination reversal learning and attentional set shifting. The effect on the former was highly specific, as it was contained within the earliest phase of reversal learning when animals typically commit more errors in favor of the previously learned reward contingency (Jones and Mishkin, 1972; Izquierdo *et al*, 2006b). MAMPH-treated rats recovered from this early-phase impairment, however, reaching criterion at a comparable rate to SAL-treated rats. There were no other differences in performance that would indicate global changes in attention or motivation such as delayed approach to food reward or stimuli.

As earlier studies report object recognition memory impairments resulting from binge doses of mAMPH, rats in this study were tested on their retention of a pretreatment discrimination problem and were found to be unimpaired. This suggests that the resulting impairment might have more to do with perturbations in behavioral flexibility and inhibitory control and less to do with deficits in learning and memory processes *per se*. It remains possible, however, that rats would have been impaired at learning a novel discrimination pair (not assessed), or that memory impairments would have manifested with higher treatment doses of mAMPH. Future studies should investigate effects of different doses and patterns of mAMPH administration on this task.

The reversal-specific impairment was also found in the ASST with slower overall learning (more trials to criterion) in mAMPH-treated rats during intradimensional and extradimensional reversals, with a trend toward the same difference in the CD reversal phase. These results are consistent with earlier reports of a reversal impairment in rats after sensitizing regimens of either amphetamine (Fletcher *et al*, 2005) or cocaine (Schoenbaum *et al*, 2004). In addition, we found no deficits in treated rats' ability to discriminate different scents and media or to shift attention from one modality to another. This finding of intact set-shifting ability stands in contrast to findings of attentional set-shifting impairments after sensitizing regimens of amphetamine (Fletcher *et al*, 2005; Featherstone *et al*, 2008). Notably, both of these studies used a 5-week, escalating-dose amphetamine regimen that results in robust behavioral sensitization to this drug, a pattern of dosing that differs markedly from the single-day binge administration used in this study. This distinction bears mentioning since sensitizing regimens of either amphetamine or mAMPH have been shown to blunt certain forms of learning by interfering with the plasticity that would otherwise occur (Kolb *et al*, 2003). The possibility exists that the differences in patterns of administration (low doses of amphetamine delivered at regular intervals across several weeks *vs* a single day, higher-dose regimen of mAMPH) elicit different results via distinct mechanisms.

Our results differ from those of Daberkow *et al* (2008), reporting large dopaminergic depletions (~55%) in the dorsomedial striatum of mAMPH-treated rats in the absence of a reversal learning impairment. Several methodological differences between the two studies could account for the lack of correspondence (eg, different doses of mAMPH administered, different treatment times used, use of a response-reversal rather than stimulus choice reversal task, different rat strain), yet one might expect that large dopaminergic depletions would produce similarly large impairments on a task such as motor response reversal learning, a striatal-dependent task. If, however, an imbalance of frontocortical-striatal systems underlies mAMPH effects on inhibitory control (described in more detail in the next section), then one could argue that smaller dopaminergic depletions in the striatum might lead to a greater imbalance, which in turn could lead to reversal learning impairments. The methods used here preclude establishing a causal relationship.

A potential noteworthy contributor to reversal learning impairments after mAMPH is stress. Elevated levels of corticosterone (CORT) are seen 1–72 h after mAMPH treatment (Herring *et al*, 2008b). Few groups have investigated stress indices such as CORT after single day, experimenter-administered mAMPH in rats, though the mechanism by which stress and mAMPH interact to bring about neurotoxicity remains an important focus of research (Szumlinski *et al*, 2001; Tata and Yamamoto, 2008). Earlier studies in rodents have reported behavioral flexibility impairments following brief, acute stressors as well as changes in dendritic morphology in areas of the frontal cortex (Izquierdo *et al*, 2006a; Holmes and Wellman, 2008).

Future investigations should identify and control for the ancillary effects of stress on mAMPH's action.

Putative Neural Mechanisms for Reversal Impairments After mAMPH Treatment

In Experiment 3, mAMPH-induced reductions in DAT were found in both dorsal and ventral divisions of the CP, with ventral CP most affected. In both magnitude and regional specificity, the pattern of reduction in striatal DAT after a single-day 4×2 mg/kg mAMPH dosing regimen agrees with the results of Belcher *et al* (2008). Such modest neurotoxicity to the ascending dopaminergic system could be sufficient to affect the intricate balance in the frontostriatal system involved in critical aspects of executive control (Robbins, 2005; Dalley *et al*, 2008). Compromised striatal circuitry may underlie the maladaptive responding reported in Experiment 1. This interpretation accords well with a recent report that medial striatal lesions in monkeys produce reversal learning impairments virtually indistinguishable from that brought about by OFC damage (Clarke *et al*, 2008; Man *et al*, 2008).

Recent positron emission tomography (PET) studies in humans provide evidence that striatal DA neurotransmission is important for performance in tasks of inhibitory control such as card sorting (Monchi *et al*, 2006). This conclusion is corroborated by evidence in animals that DA transmission in the striatum is critical for the flexible shifting of response (O'Neill and Brown, 2007; Haluk and Floresco, 2009). These more recent findings echo the long-standing theory that an intimate relationship exists between the basal ganglia network and areas like the OFC in response to changing reward contingencies (Hollerman *et al*, 2000). In addition, it has been reported that individual human differences in reversal learning performance reflect variations in baseline striatal DA synthesis capacity, measured by PET (Cools *et al*, 2009) and that different subregions of the striatum contribute to reversal learning over, for example, spatial working memory (Clatworthy *et al*, 2009). Importantly, low levels of D2 receptors in the striatum of mAMPH users have been associated with decreased metabolic activity in OFC (Volkow *et al*, 2001b; Volkow *et al*, 2004)

Both OFC and prelimbic cortex project to dorsomedial striatum in the rat (Berendse *et al*, 1992) and both frontocortical subdivisions are thought to contribute to different aspects of behavioral flexibility (Ragozzino, 2007). The pattern of behavioral impairments observed in this study—spared attentional shifting and impaired reversal learning—mimics what occurs after damage to OFC (McAlonan and Brown, 2003) but not medial frontal cortex (Birrell and Brown, 2000) damage. The evidence reviewed above supports the conclusion that the mAMPH-induced impairment of dopaminergic systems of the striatum is sufficient to impair reversal learning. However, the possibility that this binge mAMPH regimen had lasting, direct influence on cortex merits consideration. Effects of higher doses of mAMPH on integrity of neurons in somatosensory cortex (Commins and Seiden, 1986; Eisch and Marshall, 1998) have been demonstrated. However, at the doses used here, single-day binge mAMPH administration produces no loss of serotonin transporter in the cortical areas sampled (Belcher *et al*, 2008), while even higher binge mAMPH doses—sufficient to produce >50% reductions in striatal DA—result in no significant depletion of frontal cortex DA (Ohmori *et al*, 1993). Several groups have reported that a single-day binge mAMPH administration leads to altered functioning of cerebral cortex, affecting metabolism and immediate early gene expression of several cortical areas (including OFC; Pontieri *et al*, 1990; Belcher *et al*, 2009). Elsewhere, we have hypothesized that these functional changes in cerebral cortex of binge mAMPH-treated rats occur secondarily to the decreases in striatal DA, achieved through alterations in striato-nigro-thalamo-cortical loops (Marshall *et al*, 2007; Belcher *et al*, 2009). The basal ganglia-cortical loop hypothesis also provides a useful framework for interpreting the above-mentioned reports of the correspondence between diminished OFC activity and level of D2 receptors found in the brains of stimulant addicts (Volkow *et al*, 2001b). Thus, our working hypothesis is that the mAMPH-induced loss of DA in nonmotor regions of the striatum impairs the function of the OFC, providing a neurochemical and functional basis for both the observed effects on reversal learning in animals and maladaptive decision making in humans. Whether such functional impairments would be enhanced on multiple binge dose exposures has yet to be determined. The answers to such questions can be furthered by the development of valid animal models for mAMPH effects on the brain and behavioral flexibility. Such models would, in turn, go far in developing therapeutic targets for human abusers.

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DISCLOSURE

The authors declare no conflict of interest.

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