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Environmental enrichment reduces methamphetamine cue-induced reinstatement but does not alter methamphetamine reward or VMAT2 function

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Abstract

Environmental factors influence a variety of health-related outcomes. In general, being raised in an environment possessing social, sensory, and motor enrichment reduces the rewarding effects of various drugs, thus protecting against abuse vulnerability. However, in the case of methamphetamine (METH), which acts at the vesicular monoamine transporter 2 (VMAT2) to enhance dopamine release from the cytosol, previous evidence suggests that METH reward may not be altered by environmental enrichment. This study examined the influence of an enriched environment on measures of METH reward, METH seeking, and VMAT2 function. Rats were raised from weaning to adulthood in either an enriched environment (presence of social cohorts and novel objects) or an isolated environment (no cohorts or novel objects). Rats in these two conditions were subsequently tested for their acquisition of conditioned place preference (CPP), METH self-administration, maintenance of self-administration at various unit doses of METH (0.001–0.5 mg/kg/infusion), and cue-induced reinstatement. VMAT2 function in striatum from these two groups also was assessed. No significant environment effects were found in CPP or METH self-administration, which paralleled a lack of effect in VMAT2 function between groups. However, cue-induced reinstatement was reduced by environmental enrichment. Together, these results suggest that environmental enrichment does not alter VMAT2 function involved in METH reward. However, the enrichment-induced decrease in cue-induced reinstatement indicates that enrichment may have a beneficial effect against relapse following a period of extinction via a neural mechanism other than striatal VMAT2 function.

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Keywords

environmental enrichment; social isolation; methamphetamine (METH); conditioned place preference (CPP); self-administration; vesicular monoamine transporter 2 (VMAT2)

1. Introduction

Methamphetamine (METH) use remains widespread among individuals across different socio-economic strata. However, individuals from economically deprived areas are more likely to develop abuse problems with METH, as measured by use within the last month in households with a low income [1, 2], households with high unemployment, and households with parents possessing a terminal high school diploma [3]. While many factors contribute to these observations, they indicate that an individual's early environment affects risk for METH abuse.

Preclinical research has used the environmental enrichment paradigm to study the role of environmental factors on drug reward. In this paradigm, adolescent rats are raised in either an enriched condition (EC) or an isolated condition (IC). EC rats have access to social cohorts and novel objects that are replaced daily. In contrast, IC rats live in a small cage, are not handled, and are not exposed to any objects. When EC and IC rats are tested as adults, EC rats display greater conditioned place preference (CPP) to cocaine [4] and amphetamine [5], suggesting an enrichment-induced increase in drug-conditioned reward induced by multiple trials using high doses (10 mg/kg cocaine; 0.5–2.0 mg/kg amphetamine). In contrast to CPP, however, the direct reinforcing effect of stimulants is decreased by enrichment. EC rats self-administer less cocaine [4], methylphenidate [6], and amphetamine [7] at low unit doses (0.008–0.1 mg/kg/infusion cocaine; 0.056 mg/kg/infusion methylphenidate; 0.03 mg/kg/infusion amphetamine), but not at higher unit doses, relative to IC rats. EC rats also fail to reinstate extinguished responding following a dose of amphetamine (0.25 mg/kg) that reinstates responding in IC rats [8]; a higher dose of amphetamine (1 mg/kg) reinstates responding in both groups. While baseline (no drug) differences in rates of responding between EC and IC rats exist [9], these results overall indicate that enrichment may raise the primary reinforcement and reinstatement thresholds for engendering stimulant self-administration and stimulant seeking.

Studies examining METH reward in EC and IC animals have yielded mixed results that appear to contrast with results obtained with other stimulant drugs (amphetamine, cocaine, and methylphenidate). Using CPP, an effect of enrichment on METH reward has not been observed in either mice or rats [10, 11]. However, a recent study by Lü et al. [12] demonstrated differential sensitivity to METH in a nose-poke self-administration paradigm, with EC rats self-administering less METH at a unit dose of 0.03 mg/kg compared to IC rats; no differences were evident at higher unit doses. Cue-elicited reinstatement of METH seeking also was decreased in EC rats following a period of response extinction. Even though these results parallel those obtained with amphetamine, cocaine, and methylphenidate [4, 6, 7], they were obtained *after* differential rearing, rather than *during* differential rearing as in previous studies. Moreover, a limitation of this study was that the

training dose of METH (0.03 mg/kg) failed to produce reliable acquisition of self-administration in EC rats, i.e., nose-poking for METH did not differ between acquisition sessions 1 and 8. The differential rate of acquisition between EC and IC rats complicates interpretation of the subsequent assessment of extinction and reinstatement testing. One way to limit this complication is to use a high training dose in order to initially engender comparable responding in EC and IC groups [4, 6]. This procedure also allows for a more direct comparison to existing literature to determine if enrichment has a differential effect on METH reward compared to other stimulant drugs.

While all stimulant drugs of abuse increase extracellular dopamine (DA) levels in reward-relevant brain regions [13], they do so via different mechanisms of action. METH is thought to act on two main targets in the nervous system, serving as a substrate for reversing the dopamine transporter (DAT) [14, 15] and the vesicular monoamine transporter 2 (VMAT2) [16, 17]. The consequence of the combined action at these targets is increased extracellular dopamine. While VMAT2 may play a role in stimulant reward generally, evidence suggests that VMAT2 is especially important for METH reward. Behaviorally, VMAT2 inhibitors decrease self-administration of METH [18, 19] at doses that do not block either food- or cocaine-reinforced responding [18]. Neurochemically, the actions of METH at VMAT2 differ from the actions of other stimulants at this target. For example, cocaine increases [³H]dopamine uptake by VMAT2 [20], whereas METH decreases uptake [16, 21]. The ability of VMAT2 inhibitors to decrease METH self-administration specifically suggests that this molecular target may play a role in environment-dependent changes in METH self-administration.

The purpose of the current study was to extend the results provided by Lü et al. [12], except rats were tested *during* the period of differential rearing, rather than after differential rearing was terminated. METH CPP was evaluated in EC and IC rats using doses shown previously to be rewarding in our laboratory [10, 22, 23]. Additionally, METH self-administration was evaluated across various unit doses of METH (0.001–0.5 mg/kg/infusion) after first establishing reliable acquisition in both EC and IC rats using a *high training dose* of METH (0.1 mg/kg/infusion), which contrasts with the low dose used during acquisition training by Lü et al. [12]. Following a period of extinction, EC and IC rats were subsequently evaluated for cue-induced reinstatement of METH seeking. Given the role of VMAT2 in METH reward and the effect of METH at this molecular target, striatal VMAT2 function ($V_{\max}$ and K_m) also was examined in these rats. The striatum was selected for evaluation because VMAT2 deficits have been revealed in this dopamine terminal field in human METH abusers [24].

2. Materials and Methods

2.1. Subjects

Male Sprague-Dawley rats were obtained on postnatal day 21 (PND 21) from Harlan Laboratories (Indianapolis, IN) and placed immediately on a 12-h light:dark cycle (lights on at 07:00 AM) with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky and

were performed in accordance with the National Institutes of Health Guide for the Care and Use for Laboratory Animals.

2.2. Housing Conditions

Rats were assigned randomly to EC or IC conditions upon arrival. EC rats were housed with 7–11 cohorts in a stainless steel cage ($122 \times 61 \times 45.5$ cm) with 14 novel objects placed throughout. Seven of the 14 objects were changed daily. IC rats were placed singly in hanging steel cages ($17 \times 24 \times 20$ cm). These small cages allowed food and water changes, as well as waste disposal, without human contact. Rats remained in their housing conditions for the duration of the experiment (~PND 21– PND 90 for the CPP experiment, ~PND 21– PND 122 for METH self-administration and reinstatement). Separate groups of rats were used to assess METH CPP and METH self-administration.

2.3. Experiment 1: CPP

2.3.1. Apparatus—CPP was conducted in a 3-compartment chamber ($68 \times 21 \times 21$ cm; ENV-013; MED Associates, St. Albans, VT) enclosed within a sound-attenuating chamber (ENV-020 M; MED Associates). All 3 compartments differed in wall color and floor texture; the center compartment ($12 \times 21 \times 21$ cm), which was never paired with METH or saline, had gray walls and a smooth floor. One end compartment ($28 \times 21 \times 21$ cm) had white walls and a mesh floor and the other end compartment ($28 \times 21 \times 21$ cm) had black walls and a floor consisting of rods. Guillotine doors were used to confine rats to one end compartment on conditioning days.

2.3.2. Procedure—Rats from each environmental treatment group were assigned to one of 3 conditioning groups: 0.3 mg/kg METH; 1.0 mg/kg METH or saline control. The CPP procedure began on PND 70 and consisted of three phases: preconditioning, conditioning, and test. The preconditioning phase consisted of a 15-min session in which each rat was placed in the center compartment and given access to all compartments of the CPP apparatus. Time spent in each compartment was recorded and used to test for any initial compartment bias. On the day after the preconditioning phase, METH conditioning sessions were conducted in a counterbalanced, unbiased fashion; i.e. regardless of initial preference, one half of the rats received METH paired with the black compartment and saline paired with the white compartment, whereas the other half received METH paired with the white compartment and saline paired with the black compartment. Rats were placed into the appropriate compartment immediately following 0.3 mg/kg METH ($n = 9$ EC, 9 IC) or 1.0 mg/kg METH ($n = 9$ EC, 9 IC) for a 30-min conditioning session for a total of eight sessions (i.e. four METH-paired and four saline-paired). Saline control rats were treated similarly, except that they received saline in both compartments ($n = 8$ EC, 8 IC). The test for CPP was conducted on the day immediately following the last conditioning session and was identical to the preconditioning session with rats being placed in the middle compartment and given access to all chambers for 15 min. Brains were taken from these rats after their test session (see below).

2.4. Experiment 2: Self-Administration

2.4.1. Apparatus—All self-administration sessions were conducted in a standard 2-lever operant conditioning chamber (28×24×21 cm; ENV-008CT; MED Associates) equipped with a syringe pump for drug delivery (PHM-100; MED Associates). On one wall of the chamber, a lever was located on each side of a center food tray, with a white cue light located above each lever.

2.4.2. Surgical Procedures—On PND 62, a separate group of rats were surgically implanted with a chronic indwelling catheter. Prior to surgery, rats were anesthetized with ketamine (100 mg/kg, i.p.; Butler Schein, Dublin OH) and diazepam (5 mg/kg, i.p.; Hospira, Lake Forest IL). The catheter was inserted into the jugular vein, extended under the skin, and exited the body through an incision in the scalp. A cannula was attached to the end of the catheter line that protruded from the scalp and was anchored to the skull using dental acrylic and metal screws. On self-administration sessions, the cannula was attached to tubing within a leash (PHM-120; MED Associates), which was connected to a swivel (PHM-115; MED Associates) above the chamber. The tubing then exited the sound-attenuating chamber and was connected to an infusion pump (PHM-100; MED Associates). Immediately following daily self-administration sessions, rats were infused with 0.2 ml of a mixture containing 1% gentamicin (10.15 mg/ml, Abraxis BioScience, Los Angeles CA), 3% heparin (1000 USP units/ml, Abraxis BioScience, Los Angeles CA), and 96% sterile saline (0.9% NaCl).

2.4.3. METH Self-Administration: Acquisition—On PND 70, rats were trained to lever press for METH (0.1 mg/kg/infusion) using an autoshaping procedure [25, 26]. METH was administered at a constant dose adjusted for differences in body weight (within 20 g of body weight) using an equivalent infusion volume and duration across rats. For acquisition, all rats were given 7 consecutive daily 60-min autoshaping sessions. During the first 15 min of each autoshaping session, one lever (active lever, counterbalanced for side) was extended into the chamber on a random time 90-sec schedule. After 15-sec, the lever was retracted and an infusion of METH was delivered non-contingently; pressing the lever during the 15-sec extension resulted in an immediate lever retraction and infusion. Each infusion was followed by a 20-sec timeout period that was signaled by the illumination of both cue lights above the levers. This resulted in 10 total infusions of METH over the first 15 min of the autoshaping session. The second lever (inactive lever) also was extended continuously during this time; pressing on this lever had no programmed consequence. During the last 45 min of the autoshaping session, only the inactive lever was extended, with pressing having no consequences.

On the same day as each autoshaping session, rats also were given a daily 60-min FR1 METH self-administration session. For FR1 self-administration sessions, rats had continuous access to both the active and inactive levers. Responses on the active lever resulted in METH (0.1 mg/kg/infusion) delivery at the same rate and in the same volume as during the autoshaping session, while responses on the inactive lever had no programmed consequence. Each infusion was signaled by a 20-sec time out in which both cues lights were illuminated and pressing on either lever had no consequence. Unlike the autoshaping

session, both levers remained extended for the duration of the FR1 session. Autoshaping and FR1 sessions were separated by a 90–120 min interval, during which time rats were removed from the operant conditioning chamber and placed into their home cage. Rats were given both autoshaping and FR1 sessions for 7 consecutive days, after which only FR1 sessions continued for 3 additional days. Since rats received the same number of infusions during autoshaping, regardless of how many times they responded on the “active” lever, only data from FR1 sessions were included in the final analysis.

2.4.4. METH Self-Administration: Dose-response—Following the initial 10 days of acquisition training on the FR1 schedule using 0.1 mg/kg/infusion of METH, the schedule was changed to a FR3 for 3 sessions and then to a FR5 for another 3 sessions before testing began on the dose response phase of the experiment. For this phase, rats were tested with 7 doses of METH (0.001, 0.01, 0.03, 0.05, 0.1, 0.3 and 0.5 mg/kg/infusion) or saline on a FR5 schedule. Each dose was tested for 3 consecutive sessions with only the last 2 sessions included in the data analysis. Dose order was assigned randomly with the exception that saline was never the first dose assigned.

2.4.5. METH Self-Administration: Extinction—On the day following completion of the dose-response phase, rats underwent an extinction procedure that was similar to the self-administration sessions, except that the active lever no longer resulted in any programmed consequence (no infusion or cue signal). Given their history of training on the FR5 schedule in the dose-response phase, extinction sessions continued until levels of responding decreased in both EC and IC groups below that which would yield a total of 5 infusions (25 responses) averaged across 3 consecutive sessions. This criterion was achieved by using 14 extinction sessions.

2.4.6. METH Self-Administration: Cue-Induced Reinstatement—On the day following the last extinction session, reinstatement of drug seeking behavior was assessed by reintroducing the cue light that had previously signaled a METH infusion during the acquisition and dose-response phases of the experiment; however, no METH infusion was delivered. Reinstatement responding was determined in a single 60-min session.

2.5. Experiment 3: VMAT2 Function

2.5.1. Chemicals—[³H]DA (dihydroxyphenylethylamine, 3,4-[ring-2,5,6-³H]; specific activity, 28.0 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). EDTA, EGTA, L-(+) tartaric acid, HEPES, 3-hydroxytyramine (dopamine, DA), sucrose, magnesium sulfate, polyethyleneimine, sodium chloride, and ATP magnesium salt were purchased from Sigma-Aldrich (St. Louis, MO). L-Ascorbic acid and sodium bicarbonate were obtained from Aldrich Chemical Co. (Milwaukee, WI). (2*R*,3*S*,11*bS*)-2-Ethyl-3-isobutyl-9,10-dimethoxy-2,2,4,6,7,11*b*-hexahydro-1*H*-pyrido[2, 1*a*]isoquinolin-2-ol (Ro4-1284) was a gift from F. Hoffman-La Roche Ltd. (Basel, Switzerland). All other chemicals used in the assay buffers were purchased from Thermo Fisher Scientific (Waltham, MA).

2.5.2. Procedure—Kinetic analyses of [^3H]DA uptake at VMAT2 were conducted using isolated striatal synaptic vesicle preparations [27]. Between 1–5 days after CPP testing was completed (6 rats per day; one EC and one IC rat from the 0, 0.3 and 1.0 mg/kg METH dose groups), rats were decapitated and brains were dissected on an ice-cold plate to obtain the striata. Striata were homogenized with 10 up-and-down strokes of a Teflon pestle homogenizer (clearance ~ 0.003 inch) in 14 ml of 0.32 M sucrose solution. Homogenates were centrifuged (2000g for 10 min at 4°C), and then supernatants were centrifuged (10,000g for 30 min at 4°C). Pellets were resuspended in 2 ml of 0.32 M sucrose solution and subjected to osmotic shock by adding 7 ml of ice-cold MilliQ water. After 1 min, osmolarity was restored by adding 900 μl of 0.25 M HEPES buffer and 900 μl of 1.0 M potassium tartrate solution. Samples were centrifuged (20,000g for 20 min at 4°C), and supernatants were centrifuged (55,000g for 1 h at 4°C), followed by addition of 100 μl of 10 mM MgSO_4 , 100 μl of 0.25 M HEPES, and 100 μl of 1.0 M potassium tartrate solution before the final centrifugation (100,000g for 45 min at 4°C). Pellets were resuspended in 2.2 ml of assay buffer (25 mM HEPES, 100 mM potassium tartrate, 50 μM EGTA, 100 μM EDTA, 1.7 mM ascorbic acid, 2 mM ATP- Mg^{2+} , pH 7.4). Incubations were initiated by addition of 100 μl of vesicular suspension to 300 μl of assay buffer, 50 μl of inhibitor (Ro4-1284) and 50 μl of a range of [^3H]DA concentrations (0.01–1.0 μM). Nonspecific uptake was determined in the presence of Ro4-1284 (10 μM). After an incubation period of 8 min, [^3H]DA uptake was terminated by rapid filtration, and radioactivity retained by the filters was determined by liquid scintillation spectrometry (Tri-Carb 2100TR liquid scintillation analyzer; PerkinElmer Life and Analytical Sciences, Waltham MA).

2.6. Drug

(+)-Methamphetamine HCl was purchased from Sigma Aldrich (St. Louis, MO) and was dissolved in sterile saline (0.9% NaCl). In the CPP experiment, METH was injected s.c. in a volume of 1 ml/kg body weight. In the self-administration experiment, METH was injected i.v. in a constant volume of 0.1 ml/infusion, with doses adjusted by varying the drug concentration. In both CPP and self-administration experiments, METH doses were expressed as salt weight.

2.7. Data Analysis

Data from the CPP experiment were analyzed using a 2 (environment) $\times$ 2 (METH dose) $\times$ 2 (compartment) mixed model ANOVA. To determine if there was any compartment bias on the test day, time spent in the black and white compartments from the saline control group was analyzed using a 2 (environment) $\times$ 2 (compartment) mixed ANOVA. For the self-administration experiment, infusions earned and inactive lever presses for the first 7 FR1 sessions of acquisition (autoshaping phase) were analyzed separately using 2 (environment) $\times$ 7 (session) mixed model ANOVAs. Additionally, infusions earned and inactive lever presses for the last 3 FR1 sessions of acquisition (no autoshaping) also were analyzed separately using 2 (environment) $\times$ 3 (session) mixed model ANOVAs. For the dose response phase, infusions earned and inactive lever presses were analyzed using 2 (environment) $\times$ 7 (METH dose) mixed model ANOVAs; a separate Bonferroni's post hoc comparison of infusions and inactive lever presses was conducted when saline was substituted for METH. The extinction data were analyzed using a 2 (environment) $\times$ 14

(session) mixed model ANOVA. To insure rates of responding at the end of extinction did not differ between EC and IC rats, an independent samples *t*-test was used to compare the mean response rate across the last 3 days of extinction between EC and IC rats. Reinstatement was calculated as the percent change in responding on the reinstatement day from the last extinction day; these data were analyzed using an independent samples *t*-test. Since 4 rats died or lost catheter patency before the end of extinction, their data were excluded from analysis for the extinction and reinstatement phases. Data from the VMAT2 uptake assay used a 2 (environment) $\times$ 3 (CPP dose) ANOVA. Log transformed K_m values were used for statistical analysis. Bonferroni's post hoc analysis was used where appropriate. In all cases, *P* values less than 0.05 were deemed statistically significant.

3. Results

3.1. Experiment 1. METH CPP

On the preconditioning test, no significant compartment bias was found in any group (Table 1). On the postconditioning test, saline control rats did not demonstrate any compartment preference [$F(1, 14) = 4.23, p > 0.05$; $n = 8/\text{group}$, data not shown]. There also was no significant effect of environment [$F(1, 14) = .24, p > 0.05$] or interaction [$F(1, 14) = 4.16, p > 0.05$] in either EC or IC saline controls. However, on the postconditioning test day, METH-treated groups demonstrated an overall preference for the METH-paired compartment relative to the saline-paired compartment, as demonstrated by a significant main effect of compartment [$F(1, 32) = 22.93, p < 0.001$; $n = 9/\text{group}$, Figure 1]. There was also a significant main effect of environment [$F(1, 32) = 15.86, p < 0.001$]; however, there was no interaction between environment and compartment [$F(1, 32) = .23, p > 0.05$]. The main effect of environment indicated that when the data were collapsed across compartments and METH doses, EC rats spent more time overall in the end compartments compared to IC rats; the mean ($\pm$ SEM) sec in the end compartments for EC rats was 735 ($\pm$ 12) and for IC rats was 656 ($\pm$ 15). This difference was due to decreased time spent in the center gray compartment by EC rats; the mean ($\pm$ SEM) sec in the center gray compartment for EC rats was 164 ($\pm$ 12) and for IC rats was 243 ($\pm$ 15).

3.2.1. Experiment 2. METH Self-Administration: Acquisition

Both EC and IC rats learned to lever press for METH (active lever) on a FR1 schedule, as evidenced by a significant effect of session during the first 7 days of acquisition [$F(6, 84) = 4.90, p < 0.001$; $n = 7\text{--}9/\text{group}$, Figure 2A]. While there was no main effect of environment [$F(1, 14) = .25, p > 0.05$], the statistical analysis identified a significant interaction between session and environment [$F(6, 84) = 3.67, p < 0.01$]. However, Bonferroni's post hoc tests failed to identify any significant difference between groups on any individual session.

Analysis of inactive lever presses found no main effect of environment [$F(1, 14) = .79, p > 0.05$] or session [$F(6, 84) = .60, p > 0.05$], and no significant interaction [$F(6, 84) = .78, p > 0.05$; Figure 2B].

On the last 3 days of acquisition on the FR1 schedule, after autoshaping was terminated, analysis of infusions earned indicated that there was no main effect of session [$F(2, 28) = 1.45, p > 0.05$] or environment [$F(1, 14) = .22, p > 0.05$] and there was no interaction [$F(2,$

28) = 2.67, $p > 0.05$; Figure 2A]. This suggests that there was no effect of environment on acquisition overall. Analysis of inactive lever presses during the last 3 days of acquisition also yielded no main effect of session [$F(2, 28) = .75, p > 0.05$], no main effect of environment [$F(1, 14) = .55, p > 0.05$], and no interaction [$F(2, 28) = 1.06, p > 0.05$; Figure 2B]. Further, when incremented to a terminal FR5 schedule on the training dose, no differences in infusions or inactive lever presses were observed between EC and IC rats (see 0.1 mg/kg/infusion dose in Figure 3). Thus, acquisition of METH self-administration did not differ between EC and IC rats prior to the start of the dose response phase.

3.2.2. Experiment 2. METH Self-Administration: Dose Response

In the next phase of the self-administration experiment, responding at various unit doses of METH was evaluated on an FR5. Two-way mixed ANOVA with environment and METH dose as factors, using Greenhouse-Geisser correction for the violation of sphericity, revealed a main effect of dose [$F(1.70, 23.76) = 9.77, p < 0.01$; $n = 7-9/\text{group}$], but no main effect of environment [$F(1, 14) = .32, p > 0.05$] or interaction between environment and dose [$F(1.70, 23.76) = .49, p > 0.05$; Figure 3A]. The main effect of dose was due to an overall inverted U-shaped dose response curve, collapsed across EC and IC groups. Analysis of inactive lever presses also identified a significant main effect of dose [$F(6, 78) = 3.33, p < 0.01$], but no significant main effect of environment [$F(1, 13) = 1.10, p > 0.05$] or interaction [$F(6, 78) = .29, p > 0.05$; Figure 3B]. The main effect of dose on inactive lever presses was due to an overall inverted U-shaped curve, which indicates that METH had a direct effect on non-reinforced responding in both EC and IC rats.

When rats were given saline instead of METH during the dose response phase, a Bonferroni's post hoc analysis revealed a significant difference between EC and IC rats, $p < 0.05$; the mean ($\pm$ SEM) number of saline infusions in EC rats was 5.0 ($\pm$ 1.1) and in IC rats was 10.6 ($\pm$ 1.9). This finding indicates that EC rats showed a greater loss of perseveration of non-reinforced responding (i.e., more extinction) than IC rats (data not shown).

3.2.3. Experiment 2. METH Self-Administration: Extinction

Both EC and IC rats decreased responding across the 14 extinction sessions. There was a significant main effect of extinction session [$F(13, 130) = 11.69, p < 0.001$; $n = 5-7/\text{group}$] and a main effect of environment [$F(1, 10) = 6.92, p < 0.05$], but no significant interaction (Figure 4). The main effect of session was due to an overall decrease in responding across sessions, collapsed across groups; the main effect of environment was due to an overall lower rate of responding in EC rats compared to IC, collapsed across sessions. Importantly, however, the average responding on the last 3 extinction sessions did not significantly differ between EC and IC rats [$t(10) = -1.43, p > 0.05$], indicating that both groups displayed a similar loss of responding by the end of extinction.

3.2.4. Experiment 2. METH Self-Administration: Cue-Induced Reinstatement

Cue-induced reinstatement was calculated as the average percent change in lever pressing from the last extinction day. An independent samples t -test found a significant difference between EC and IC rats [$t(10) = -2.35, p < 0.05$; $n = 5-7/\text{group}$] in responding during cue-

induced reinstatement (Figure 5). Thus, EC rats showed less METH seeking relative to IC rats.

3.3. Experiment 3. VMAT2 Function

To determine the effect of different doses of METH (used in CPP) on VMAT2 function in EC and IC rats, kinetic analysis of [3 H]DA uptake into isolated striatal synaptic vesicles was performed (Table 2). Two-way ANOVAs revealed no significant main effects of environment or METH dose or interactions for either $V_{\max}$ [$F(2, 31) = 0.26, p > 0.05$] or K_m [$F(2, 31) = 0.51, p > 0.05, n = 6-7/\text{group}$], indicating no change in VMAT2 function in response to the environment or METH treatment.

4. Discussion

The current study evaluated METH reward using CPP and self-administration in rats raised in either an enriched or isolated environment and then tested while housed in these environments. Despite the various previous studies demonstrating a protective effect of environmental enrichment on reward produced by cocaine, methylphenidate or amphetamine [4–7], the current study failed to identify environment-induced differences in METH reward. EC and IC rats demonstrated similar CPP using either 0.3 or 1.0 mg/kg of METH. Similarly, with METH self-administration using a high training dose (0.1 mg/kg/infusion), similar infusion rates were engendered on a FR1 schedule in EC and IC rats. Further, during the dose response phase, no significant differences in METH intake between EC and IC occurred at any unit dose tested (0.001–0.5 mg/kg/infusion). However, when saline was substituted for METH, EC rats displayed less persistence in responding compared to IC rats. This difference also was evident during the extinction phase when both the light cue (time out signal) and METH infusion were omitted. Importantly, however, there were no differences between EC and IC rats at the end of 14 extinction sessions. When the light cue was reintroduced, EC rats showed less reinstatement responding than IC rats. This enrichment-induced decrease in cue-induced reinstatement does not likely reflect a difference in VMAT2 function, as kinetic analysis of [3 H]DA uptake into striatal vesicles revealed no differences in $V_{\max}$ or K_m between EC and IC rats exposed to METH during CPP.

The finding that EC and IC rats both developed similar CPP at the 0.3 and 1 mg/kg doses of METH is consistent with previous work showing no difference in METH CPP between enriched and standard housed mice [11] or between EC and IC rats [10]. Thus, in contrast to the enrichment-induced increase in CPP produced by amphetamine and cocaine [4, 5], this effect does not generalize to METH.

In related work, the effect of differential rearing on METH CPP was determined following pretreatment with neurotoxic doses of METH [10]. In that work, EC and IC rats were first pretreated with high doses of METH (10 mg/kg, four injections at 2 h intervals) or saline at 55 days of age and then were tested for CPP using either 0.3 or 1.0 mg/kg of METH beginning at 63 days of age. Consistent with the current results, no differences in METH CPP were observed between EC and IC rats given control (no high doses of METH) pretreatment. However, EC rats pretreated with high doses of METH showed enhanced CPP

compared to IC rats when conditioned with METH (0.3 mg/kg). The discrepancy between the previous study and the current study is likely attributed to pretreatment with repeated high doses of METH, as high dose METH pretreatment causes dopamine depletion [11, 23, 28, 29]. Although EC and IC rats showed a similar dopamine depletion with high dose METH pretreatment, metabolite levels were higher in EC rats [10], suggesting a differential effect on presynaptic dopamine function which may be related to the environment-induced alteration in METH CPP. However, a more recent study found no environment-dependent change in either METH neurotoxicity or CPP in mice [11]. Further work is needed to determine the conditions under which enrichment has a protective effect, if any, against the neurotoxic and behavioral consequences of high dose METH exposure.

One unanticipated finding in the CPP experiment was the decrease in time spent in the center gray compartment in EC rats relative to IC rats on the test day. This effect was observed regardless of drug conditioning treatment. Since the center gray compartment was smaller than the end compartments, perhaps IC rats showed a greater preference than EC rats because it was a “protected” space. Consistent with this possibility, IC rats show greater anxiety-like signs compared to EC rats in the elevated plus maze [30]. Alternatively, since rats were exposed to only the two end compartments during conditioning, IC rats may have displayed a greater preference than EC rats due to the relative novelty of the center gray on the test day. Supporting this latter possibility, novel visual stimuli are more reinforcing in IC rats than EC rats [31].

To assess the primary reinforcing effects of METH directly, self-administration was evaluated in EC and IC rats. Using the current autoshaping procedure, EC and IC rats acquired METH self-administration similarly across an incrementing FR1-FR5 schedule. Since all rats received the same number of infusions during autoshaping, regardless of how many times they responded on the “active” lever, acquisition of FR1 responding should not have been differentially affected by the autoshaping procedure in EC and IC rats. In addition, with the FR5 schedule, no differences between EC and IC rats were obtained across a wide range of unit doses (0.001–0.5 mg/kg/infusion). These findings contrast with previous work showing reduced self-administration of methylphenidate [6], cocaine [4], and amphetamine [7] in EC rats compared to IC rats, especially at low unit doses. The current results also contrast with findings from a previous report by Lü et al. [12] showing that, following termination of the differential housing conditions, EC rats self-administer less METH than IC rats during acquisition. However, in addition to the difference in housing conditions maintained during testing, Lü et al. [12] used a training dose of 0.03 mg/kg/infusion, whereas the current experiment used a training dose of 0.1 mg/kg/infusion. The higher training dose used here was selected specifically to minimize the potential for differential rates of acquisition prior to dose response determination.

Despite the lack of effect during acquisition and dose response phases, the current report shows that EC rats display less responding than IC rats during extinction. It is possible that the non-contingent METH infusions during autoshaping sessions may have slowed the overall rate of extinction and this may have affected responding differentially in EC and IC rats. Importantly, however, extinction training was continued for 14 sessions, at which point both EC and IC rats displayed fewer than 25 responses (which would have yielded fewer

than 5 infusions on an FR5) across 3 consecutive sessions. This allowed for the determination of reinstatement when extinguished responding in EC and IC rats was similar. When subsequently tested for cue-induced reinstatement, EC rats again showed less responding than IC rats. Faster extinction rates in EC rats have been observed for other psychostimulants [4, 6] and both faster extinction and reduced cue-induced reinstatement in EC rats are consistent with Lü et al. [12]. The enrichment-induced decrease in METH seeking also is consistent with results showing that EC rats attribute less incentive value to a lever associated previously with reward compared to IC rats [32]. Thus, the specific enrichment-induced decrease in response perseveration and cue-induced reinstatement observed here suggests that environmental enrichment protects specifically against METH seeking induced by cue exposure following abstinence, without directly altering the rewarding effect of METH.

Given the role of VMAT2 in METH reward [18], kinetic analysis of VMAT2 also was examined in EC and IC rats. Maximum velocity of [^3H]DA uptake (V_{max}) and K_m values obtained from striatal vesicles were similar between EC and IC rats. These neurochemical results parallel the similar acquisition of METH CPP and METH self-administration observed in EC and IC rats, which would be predicted if METH reward is dependent primarily on actions at VMAT2. These findings contrast with previous work showing that enrichment decreases self-administration of DAT inhibitors such as cocaine and methylphenidate at low unit doses [4, 6] and decreases DAT function in prefrontal cortex [33, 34]. These findings together suggest that enrichment may preferentially decrease the rewarding effect of DAT inhibitors, but not VMAT2 inhibitors. One caveat to this conclusion is that amphetamine also serves as a substrate at VMAT2 [35] and enrichment reduces amphetamine self-administration [7]. While both METH and amphetamine serve as substrates at VMAT2, these stimulants also are known to have differential effects on dopamine and glutamate levels, as well as norepinephrine transporters, in prefrontal cortex [36, 37]. Further work is needed to elucidate the neural changes underlying the environment-dependent responses to stimulant drugs with different mechanisms of action.

While VMAT2 plays a role in METH reward, its role in cue-induced reinstatement is less clear. To date, only one study has examined the role of VMAT2 function in cue-induced reinstatement of METH seeking [38]. In that study, a novel inhibitor of VMAT2 (GZ-793A) decreased cue-induced reinstatement without decreasing responding when given alone. In the current study, EC rats displayed reduced cue-induced reinstatement compared to IC rats, despite having no change in VMAT2 function. While this finding may indicate that VMAT2 is *not* involved in environment-dependent changes in METH seeking, at least two important methodological details prevent this firm conclusion. First, METH seeking and VMAT2 function were determined using different groups of rats. Second, VMAT function was determined only in vesicles from striatum. While both METH self-administration and cue-induced reinstatement may involve actions at VMAT2 [18, 19, 38], the brain areas responsible for these behaviors may differ. In particular, it is known that extinction and reinstatement of METH seeking recruits areas outside the ventral striatum, including prefrontal cortex, hippocampus and amygdala [39–42]. Interestingly, brain areas recruited for cue-induced METH seeking differ from those involved in cue-induced cocaine seeking

[42]. Ultimately, whether action at VMAT2 in these specific brain regions is necessary for METH seeking remains to be determined.

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Highlights

Enriched and isolated rats developed similar methamphetamine CPP.

Acquisition and dose-response of methamphetamine self-administration did not differ between enriched and isolated rats.

Enriched rats demonstrated reduced cue-induced reinstatement compared to isolated rats.

No differences were found in striatal VMAT2 function between enriched and isolated rats.

Figure 1. Effect of environmental enrichment on METH CPP

Data presented as mean ($\pm$ SEM) sec spent in the METH-paired and saline-paired compartments in EC and IC rats conditioned with either 0.3 or 1 mg/kg of METH.

Figure 2. Effect of environmental enrichment on acquisition of METH self-administration
A. Mean (± SEM) infusions earned by EC and IC rats during the FR1 self-administration session following autoshaping on sessions 1–7 and without autoshaping on sessions 8–10. **B.** Mean (± SEM) inactive lever presses by EC and IC rats during the self-administration session following autoshaping on sessions 1–7 and without autoshaping on sessions 8–10. * $p < 0.05$ main effect of session

Figure 3. Effect of environmental enrichment on the dose response for METH self-administration

A. Mean ($\pm$ SEM) infusions earned by EC and IC rats across various unit doses of METH.

B. Mean ($\pm$ SEM) inactive lever presses by EC and IC rats across various unit doses of METH. Note that since rats were on a FR5 schedule in this phase of the experiment, the number of active lever presses can be estimated by multiplying the number of infusions plotted in Panel A by 5.

Figure 4. Effect of environmental enrichment on extinction of METH self-administration
Data are presented as mean ($\pm$ SEM) lever presses on the previously active lever by EC and IC rats across 14 extinction sessions. n.s.: no significant difference between EC and IC.

Figure 5. Effect of environmental enrichment on cue-induced reinstatement

Data are presented as the mean ($\pm$ SEM) percent change in lever presses on the previously active lever during cue-induced reinstatement relative to the last day of extinction in EC and IC rats. Percent change in infusions earned was calculated as: $[(\text{Number of lever presses on last day of extinction} / \text{number of lever presses after cue presentation}) \times 100]$. * $p < .05$ compared to IC.

Table 1
Time spent in each compartment of CPP apparatus during preconditioning for each treatment group

Data are presented as mean ($\pm$ SEM) sec spent in each compartment.

METH dose (mg/kg)	EC		IC	
	Black	White	Black	White
0	399 ($\pm$ 58) sec	371 ($\pm$ 64) sec	385 ($\pm$ 29) sec	352 ($\pm$ 23) sec
0.3	329 ($\pm$ 43) sec	361 ($\pm$ 50) sec	356 ($\pm$ 28) sec	360 ($\pm$ 22) sec
1.0	366 ($\pm$ 25) sec	353 ($\pm$ 34) sec	363 ($\pm$ 26) sec	334 ($\pm$ 46) sec

Table 2
 $V_{\max}$ and K_m values for [^3H]DA uptake into striatal vesicles

Data presented as mean ($\pm$ SEM) $V_{\max}$ or K_m from rats used in the METH CPP experiment.

METH dose (mg/kg)	EC		IC	
	$V_{\max}$ (pmol/min/mg)	K_m (nM)	$V_{\max}$ (pmol/min/mg)	K_m (nM)
0	79.1 $\pm$ 9.4	156 $\pm$ 22.3	69.8 $\pm$ 9.1	193 $\pm$ 53.7
0.3	73.3 $\pm$ 11.9	109 $\pm$ 15.5	60.5 $\pm$ 7.3	166 $\pm$ 32.1
1.0	66.0 $\pm$ 11.8	132 $\pm$ 39.7	66.8 $\pm$ 6.8	201 $\pm$ 33.9