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ORIGINAL INVESTIGATION

Impact of mGluR5 during amphetamine-induced hyperactivity and conditioned hyperactivity in differentially reared rats

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Abstract
Rationale 3-((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP) is a metabotropic glutamate receptor 5 (mGluR5) antagonist that may alter drug sensitivity in differentially reared rats due to its involvement in the psychostimulant reward pathway and plasticity.
Objectives The purpose of this study was to assess the effects of MTEP on acute amphetamine-induced hyperactivity, conditioned hyperactivity, and sensitization.
Methods Rats were reared in an enriched (EC), isolated (IC), or standard (SC) condition after which rats were either administered MTEP (1.0 mg/kg, ip) or saline prior to an acute (0.5 or 1.0 mg/kg, sc) or repeated (0.3 mg/kg, sc) amphetamine exposure. Rats undergoing repeated amphetamine exposure were administered MTEP prior to conditioned hyperactivity and sensitization tests.
Results EC and SC rats administered with MTEP prior to acute amphetamine demonstrated attenuated amphetamine-induced locomotor activity compared to controls, while IC rats administered MTEP following repeated amphetamine exposure demonstrated attenuated amphetamine-induced locomotor activity. Interestingly, MTEP treatment only altered conditioned hyperactivity in EC rats, as MTEP pretreatment resulted in conditioned hyperactivity in EC rats while conditioned hyperactivity was not observed in EC rats pretreated with saline.
Conclusions Glutamatergic pathways are altered during differential rearing, which differentially alters the role of mGluR5 in EC, IC, and SC rats when administered psychostimulant acutely versus repeatedly. These findings suggest that differential rearing alters glutamatergic function, which reduces sensitivity to psychostimulants.

Keywords mGluR5 · Environmental enrichment · Conditioned hyperactivity · Sensitization · Amphetamine · Rat

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Several environmental factors influence drug abuse, and one environmental factor that appears to influence drug abuse during adolescence is differential rearing. The enrichment paradigm is used to study the effects of differential rearing on drug abuse, and typically consists of three environmental contexts, an enriched condition (EC), an isolated condition (IC), and a standard condition (SC; Renner and Rosenzweig 1987). Typically, rearing conditions differ in the number of rats housed together, the amount of handling, and the number and type of novel objects in the cage (Bardo and Dwoskin 2004; Renner and Rosenzweig 1987). While numerous studies have demonstrated that environmental enrichment protects against both the acute and chronic effects of a variety of drugs of abuse (Bardo and Dwoskin 2004; Simpson and Kelly 2011; Stairs and Bardo 2009), the neurobiological mechanism for this protective effect remains unclear.

Rearing rats in EC, IC, or SC contexts alters amphetamine-induced hyperactivity and sensitization in a dose-dependent manner. EC rats display greater amphetamine-induced hyperactivity than IC rats following exposure to a moderate (1.0 mg/kg) dose of amphetamine, but no differences are observed following a low unit dose (0.1 or 0.3 mg/kg; Bardo et al. 1995; Bowling and Bardo 1994). Differential rearing also alters amphetamine-induced sensitization in a dose-

dependent manner. A high dose of amphetamine produces sensitization in EC and IC rats, but a low dose only produces sensitization in IC rats (Bardo et al. 1995). Consistent with the observation that differential rearing alters amphetamine-induced hyperactivity and sensitization in a dose-dependent manner, changes in learning and memory (Renner and Rosenzweig 1987), and specifically Pavlovian conditioning (Barbelivien et al. 2006; Duffy et al. 2001; Woodcock and Richardson 2000) are also dose dependent. Differences in conditioned place preference (CPP) between EC and IC rats are apparent at low to moderate doses of psychostimulants, but not at high doses (Bowling and Bardo 1994; Solinas et al. 2008).

Differential rearing causes several neuroanatomical changes in EC compared to IC and SC rats that may contribute to the behavioral response to psychostimulants (Renner and Rosenzweig 1987; Simpson and Kelly 2011). As glutamate influences both synaptic transmission and plasticity (Giorgetti et al. 2001; Melendez et al. 2004; Wolf 1998; Wolf and Xue 1999), it may be involved in the synaptic changes associated with differential rearing (Altschuler 1979; Duffy et al. 2001; Green and Greenough 1986). EC rats have greater levels of metabotropic glutamate receptor 5 (mGluR5) dimers in the prefrontal cortex (PFC) compared to IC rats, though there are no differences in mGluR5 monomers (Melendez et al. 2004). In the nucleus accumbens (NAcc), EC rats have reduced NMDA receptors compared to IC rats, but there are no differences in AMPA receptors (Wood et al. 2005). Rahmen and Bardo (2008) demonstrated that EC rats have greater levels of glutamate compared to IC rats in the NAcc following amphetamine, but glutamate levels do not differ between rearing groups following saline. While it is clear that differential rearing alters glutamatergic systems, the role of these changes in mediating psychostimulant sensitivity is not clear.

Metabotropic glutamate receptor 5 is a viable candidate for contributing to the differences in drug sensitivity between EC and IC rats due to its involvement in the psychostimulant reward pathway, plasticity, and differential rearing (McGeehan and Olive 2003; Rahman and Bardo 2008; Schwendt and McGinty 2007; van Praag et al. 2001). Both the stimulant function of amphetamine and the reinforcing effects of amphetamine are reduced by two different mGluR5 antagonists, 3-((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP; Kumaresan et al. 2009; Martin-Fardon et al. 2009) and 2-Methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; Bäckström and Hyytiä 2007). MTEP attenuates methamphetamine-induced reinstatement (Gass et al. 2009), as well as methamphetamine (Gass et al. 2009) and cocaine (Hao et al. 2010; Martin-Fardon et al. 2009) self-administration in a dose-dependent manner. Importantly, MTEP does not attenuate spontaneous locomotor activity or lever pressing for food (Gass et al. 2009). Despite the effects of MTEP on psychostimulant self-administration, it is not clear if it attenuates acute psychostimulant-induced hyperactivity. A recent study suggests that MTEP does not attenuate the acute psychostimulant-induced hyperactivity effects of a high dose of cocaine (30 mg/kg; Veeneman et al. 2011). The role of mGluR5 in psychostimulant-induced sensitization is also unclear. Dravolina et al. (2006) did not observe an effect of MTEP on cocaine-induced behavioral sensitization (10 mg/kg); however, rats were not rested for the standard 1–4 weeks following training. In contrast, a recent experiment rested the rats for 3 weeks following training and observed that MTEP did attenuate cocaine-induced sensitization when a high dose of cocaine (30 mg/kg) was administered (Veeneman et al. 2011).

Metabotropic glutamate receptor 5 also contributes to a variety of Pavlovian-conditioned processes related to drug abuse. MTEP and MPEP both attenuate cue-induced reinstatement (Martin-Fardon et al. 2009). However, the effects of MTEP and MPEP on cocaine-induced CPP are inconsistent. Several studies have suggested that MPEP blocks the acquisition of cocaine-induced CPP (Herzig and Schmidt 2004; McGeehan and Olive 2003). In contrast, recent studies suggest that MPEP enhances the acquisition of cocaine-induced CPP when low doses of cocaine are administered (Rutten et al. 2010) and neither MPEP or MTEP block the acquisition of cocaine-induced CPP when moderate doses of cocaine are administered (Rutten et al. 2010; Veeneman et al. 2011).

The current experiment was designed to provide initial support for our hypothesis that changes in the glutamatergic system, specifically mGluR5, contributes to the ability of environmental enrichment to protect against drug use. We investigated the effect of mGluR5 antagonism on acute amphetamine-induced hyperactivity, as well as the expression of amphetamine-induced conditioned hyperactivity and sensitization in differentially reared rats. To determine the role of mGluR5 in both amphetamine-induced contextual conditioning and sensitization, we have included a group of standard-housed rats in the current experiments. We hypothesized that MTEP would attenuate amphetamine-induced hyperactivity, conditioned hyperactivity, and sensitization when moderate, but not high doses, of amphetamine were administered. We also hypothesized that differential rearing would alter the effects of MTEP due to the changes that arise in glutamatergic systems as a result of differential rearing.

Method

Subjects

Male Sprague Dawley rats were obtained from Charles River (Portage, MI, USA), and housed in one of three

environments described below. Rats had ad libitium access to food and water throughout the experiment. The colony was maintained at 22°C and humidity ranged from 30% to 45% with a 12-h light–dark cycle (lights on from 0700 to 1900 hours). Behavioral testing was conducted during the light portion of the cycle. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee at Kansas State University, and complied with NIH guidelines (National Research Council 1996).

Differential rearing

Rats arrived in the lab at 21 days of age and were randomly assigned to one of three environmental rearing conditions. EC rats were reared in groups of 12 in large metal cages (60×120×45 cm) that were lined with paper pulp bedding. Fourteen novel objects (children's toys and PVC pipe) were placed in each cage. Seven of the novel objects were changed daily, and all novel objects were changed twice weekly. EC rats were also handled daily throughout rearing. IC rats were reared individually in hanging wire cages (17×24×20 cm). IC cages had wire mesh on the front and bottom, and solid sides. IC rats were not handled during the rearing period. SC rats were housed in pairs in standard shoebox cages (20×43×20 cm). SC cages were lined with paper pulp bedding and had wire tops. SC rats were only handled during the scheduled weekly cage change. Rats were reared in their respective conditions for 30 days and remained in their housing condition for the duration of the experiment.

Apparatus

Experiments were conducted using six locomotor chambers. The chambers were 40.64×40.64×40.64 cm (Coulbourn Instruments, TruScan 2.01) and had clear plexiglass walls and a stainless steel floor covered with either pine-chip bedding or pelleted paperchip bedding. Photobeams were arranged in a 16 (*X*-axis) photocell array, spaced 2.54 cm apart (center to center). Locomotor activity was measured by recording the total distance traveled (centimeter). Throughout the session a 70-db white noise was generated to mask background noise.

Drugs

D-amphetamine (Sigma Aldrich Dallas, TX, USA) was dissolved in 0.9% saline (0.3, 0.5, and 1.0 mg/kg, 1.0 mg/mL) and injected subcutaneously. MTEP (Tocris Bioscience, MO, USA and Ascent Scientific, Princeton, NJ, 08450) was dissolved in 0.9% saline (1.0 mg/kg, 1.0 mg/mL) and injected intraperitoneally. The current study used the mGluR5 antagonist MTEP as it is more potent and specific than MPEP (Cosford et al. 2003; Mathiesen et al. 2003).

Behavioral procedures

Experiment 1: acute amphetamine

Following 30 days of rearing, EC, IC, and SC rats (n=36; 12 per group) underwent a 1-h habituation session. Rats were transferred to the locomotor room and all rats received a saline injection (subcutaneously, s.c.) immediately prior to being placed in the locomotor chamber. Following the initial habituation session, rats underwent four test sessions. Rats received a low dose of amphetamine (0.5 mg/kg) across the first two test sessions and a high dose of amphetamine (1.0 mg/kg) across the last two test sessions. Prior to the test session, rats received an MTEP (1 mg/kg, intraperitoneally, i.p.) or saline injection. Thirty minutes later (Palmatier et al. 2008), rats received either an amphetamine (0.5 or 1.0 mg/kg, s.c.) or saline challenge injection immediately prior to being placed in the locomotor chamber for 1 h. Rats were assigned to MTEP and amphetamine treatment groups in a counterbalanced manner. Thus, over the four tests, each rat was administered each of the four pairings (MTEP–AMP, Saline–AMP, MTEP–Saline, or Saline–Saline). Rats rested in their respective home cages for several days following each testing session. Intervening between each test session, rats received an additional habituation session resulting in two habituation sessions for each dose tested.

Data analysis

The total distance traveled (centimeter) was analyzed separately across the two test sessions for each dose of amphetamine tested. During each block of test sessions, the total distance traveled (centimeter) was analyzed using a 2×3×4 mixed factorial analysis of variance (ANOVA) for each amphetamine dose. Environmental condition (EC, IC, SC) and treatment order (four possible orders) served as the between-subjects factors and test session served as the within-subjects factor. Once it was confirmed that the order of MTEP and amphetamine administration did not alter responding across the test sessions, separate 2×2×3 mixed factorial ANOVAs were performed for each amphetamine dose. Environmental condition and MTEP treatment served as between-subjects factors. Session served as a within-subjects factor. The total distance traveled (centimeters) during the block of two habituation sessions for each dose was analyzed using a 2×2×3 mixed factorial ANOVA to ensure that spontaneous locomotor activity did not change across the test sessions. Multiple comparisons were used to

probe any significant interactions. Alpha was deemed significant at $p<0.05$.

Experiment 2: repeated amphetamine

Acquisition

Following 30 days of rearing, EC, IC, and SC naïve rats ($n=108$) were assigned to either paired, unpaired, or control groups ($n=36$ per group) in a counterbalanced manner. Experiments were performed in three separate groups, with each group consisting of rats from every rearing and treatment condition. Rats underwent a 1-hr locomotor session for five sessions on alternating days. Rats rested in their home cages on alternating days. Paired rats received an amphetamine injection (0.3 mg/kg, s.c.) prior to being placed in the locomotor chamber, and a saline injection in their home cage on alternating days. Unpaired rats received a saline injection prior to being placed in the locomotor chamber, and amphetamine on alternating days in their home cage. Control rats received saline in both locations. During acquisition sessions, no MTEP was administered.

Conditioned hyperactivity test

After acquisition, rats underwent a conditioned hyperactivity test. Rats were administered an MTEP (1 mg/kg, i.p.) or saline injection. Thirty minutes later, all rats received a saline injection immediately prior to being placed in the locomotor chamber for a 1-h session.

Sensitization training

Rats received five additional 1-h training sessions in the locomotor chambers during which they received amphetamine or saline injections. Procedures and drug treatments were identical to those during acquisition. Following sensitization training, rats rested in their home cages for 14 days.

Sensitization test

After the 2-week rest period, rats received an MTEP (1 mg/kg, i.p.) or saline injection 30 min prior to the sensitization test. Treatment groups were pseudorandomly assigned, to ensure that rats only received one MTEP injection during the experiment. Immediately prior to being placed in the locomotor chamber for a 1-h session all rats received an amphetamine (0.3 mg/kg, s.c.) challenge injection. In the current study, sensitization is defined as greater amphetamine-induced locomotor activity following repeated psychostimulant administration compared to repeated saline administration.

Data analysis

The total distance traveled (centimeters) during each training phase was analyzed using a 3×3×5 mixed subjects ANOVA. Environmental condition and amphetamine treatment group served as between-subjects factors. Session served as a within-subjects factor. Multiple comparisons were used to probe any significant interactions. The conditioned hyperactivity and sensitization tests were analyzed using two separate between-subjects ANOVAs. For both between-subjects ANOVAs, environmental condition, amphetamine treatment group, and MTEP treatment group served as between-subjects factors. Multiple comparisons were used to probe any significant interactions. Alpha was deemed significant at $p<0.05$.

Results

Experiment 1: acute amphetamine

Low dose (0.5 mg/kg amphetamine)

To ensure that there were no changes in spontaneous locomotor activity across the two low-dose test sessions, habituation sessions were compared using a 2×2×3 mixed factorial ANOVA. The results confirmed that there were no changes in the total distance traveled across habituation sessions. However, results did indicate a main effect of environmental condition [$F(2, 30)=21.23$, $p<0.001$]. Across the habituation sessions, EC rats had a significantly less locomotor activity than both IC [Fs$(1, 30)>4.92$, ps<0.05] and SC [Fs$(1, 30)>8.63$, ps<0.05] rats.

In order to confirm that the order of MTEP and amphetamine administration across the two low-dose test sessions did not affect locomotor activity, we conducted a 2×3×4 mixed factorial ANOVA. While main effects for session [$F(1, 24)=173.86$, $p<0.001$] and environmental conditions [$F(2, 24)=25.50$, $p<0.001$] were observed, there was no effect of test order. This indicates that the order of MTEP administration did not alter responding across sessions. Therefore, the treatment order was not included in subsequent ANOVAs.

Results of a 2×2×3 mixed factorial ANOVA indicate that differential rearing alters the ability of MTEP to significantly attenuate amphetamine-induced hyperactivity (Fig. 1). An ANOVA revealed the main effects for session [$F(1, 30)=135.09$, $p<0.001$] and environmental conditions [$F(2, 30)=22.04$ $p<0.001$], as well as a significant session×MTEP treatment interaction [F $(1, 30)=31.35$, $p<0.001$].

Simple effects analyses indicate that MTEP attenuated amphetamine-induced hyperactivity in EC [$F(1, 30)=5.71$, $p<0.05$] and SC [$F(1, 30)=22.04$, $p<0.05$] but not IC rats. MTEP pretreatment did not have an effect on the total

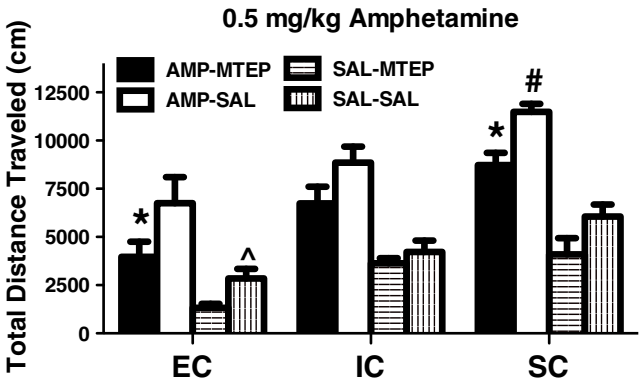

Fig. 1 Total locomotor distance (centimeters) traveled in EC, IC, and SC rats following pretreatment with MTEP (1.0 mg/kg) or saline and an acute injection of amphetamine (0.5 mg/kg) or saline. *$p<0.05$ significant difference between MTEP and saline rats. #$p<0.05$ significant difference between SC and IC/EC amphetamine-saline rats. ^$p<0.05$ significant difference between EC and SC saline–saline rats

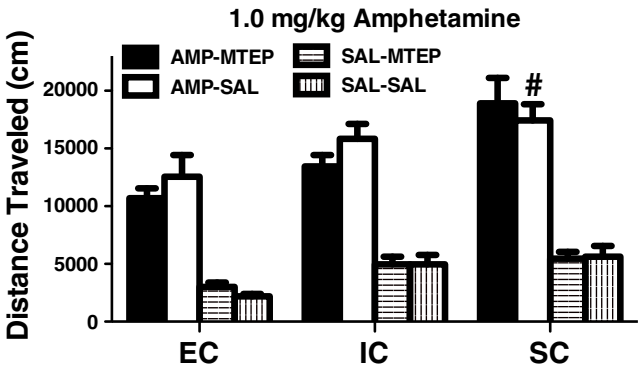

Fig. 2 Total locomotor distance (centimeters) traveled in EC, IC, and SC rats following pretreatment with MTEP (1.0 mg/kg) or saline and an acute injection of amphetamine (1.0 mg/kg) or saline. #$p<0.05$ significant difference between SC and EC amphetamine-saline rats

distance traveled in rats administered saline. In the absence of MTEP pretreatment, SC rats displayed greater amphetamine-induced hyperactivity than both IC [$F(1, 30)=5.09$, $p<0.05$] and EC [$F(1, 30)=16.44$, $p<0.05$] rats. Additionally, SC saline–saline rats had greater locomotor activity than EC rats [$F(1, 30)=5.66$, $p<0.05$].

High dose (1.0 mg/kg amphetamine)

To ensure there were no changes in spontaneous locomotor activity across the two high-dose test sessions, habituation sessions were compared using a 2×2×3 mixed factorial ANOVA. Results confirmed that there were no changes in the total distance traveled across the habituation sessions. However, results did indicate a main effect of environmental condition [$F(2, 30)=15.63$, $p<0.001$]. Across the habituation sessions, EC rats had significantly less locomotor activity than both IC [Fs$(1, 30)>7.94$, ps<0.05] and SC [Fs$(1, 30)>11.46$, ps<0.05] rats.

In order to confirm that the order of MTEP and amphetamine administration across the two high-dose test sessions did not affect locomotor activity, we conducted a 2×3×4 mixed factorial ANOVA. Main effects of session [$F(1, 24)=457.83$, $p<0.001$] and environmental condition [$F(2, 24)=15.59$, $p<0.001$] were observed. There was not a main effect of test order, indicating that the order of MTEP and amphetamine administration did not alter responding across sessions. Therefore, the treatment order was not included in subsequent ANOVAs.

Results of a 2×2×3 mixed factorial ANOVA indicate that MTEP does not significantly attenuate amphetamine-induced hyperactivity when a higher dose of amphetamine is administered (Fig. 2). There were main effects for session [$F(1, 30)=319.48$, $p<0.001$] and environmental conditions [$F(2, 30)=14.14$, $p<0.001$], as well as a significant interaction between session and environmental conditions [$F(1, 30)=3.58$, $p<0.05$]. Simple effect analyses only indicated a significant difference in amphetamine-induced hyperactivity between EC and SC rats pretreated with saline [$F(1, 30)=8.95$, $p<0.05$]. MTEP pretreatment had no effect on the total distance traveled in rats administered a high dose of amphetamine or saline.

Experiment 2: repeated amphetamine

Acquisition

During acquisition sessions, rats received amphetamine or saline treatments prior to locomotor sessions. No MTEP was administered during this phase. Overall, results of acquisition revealed that locomotor activity was attenuated in EC compared to IC and SC rats. Additionally, paired rats within each environmental condition demonstrated greater locomotor activity than unpaired and control rats.

A 3×3×5 ANOVA showed a main effect of environmental condition [$F(2, 99)=84.93$, $p<0.001$] and a main effect of amphetamine treatment [$F(2, 99)=169.99$, $p<0.001$]. Analysis also revealed a session×environmental condition [$F(8, 396)=4.20$, $p<0.001$] and a session×amphetamine treatment interaction [$F(8, 396)=28.76$, $p<0.001$].

For all amphetamine treatment conditions, locomotor activity was attenuated in EC compared to IC and SC rats during acquisition. During all 5 sessions of acquisition, EC paired rats demonstrated attenuated locomotor activity compared to IC paired [Fs$(1, 396)>6.36$, ps<0.05] and SC paired [Fs$(1, 396)>39.17$, ps<0.001] rats (Fig. 3a). Results revealed that IC paired rats had attenuated locomotor activity compared to SC paired rats during sessions 1, 3, and 5 [Fs$(1, 396)>6.13$, ps<0.05]. In unpaired and control rats administered with saline immediately prior to the session, EC rats tended to have less locomotor activity than IC and SC rats (Fig. 3b).

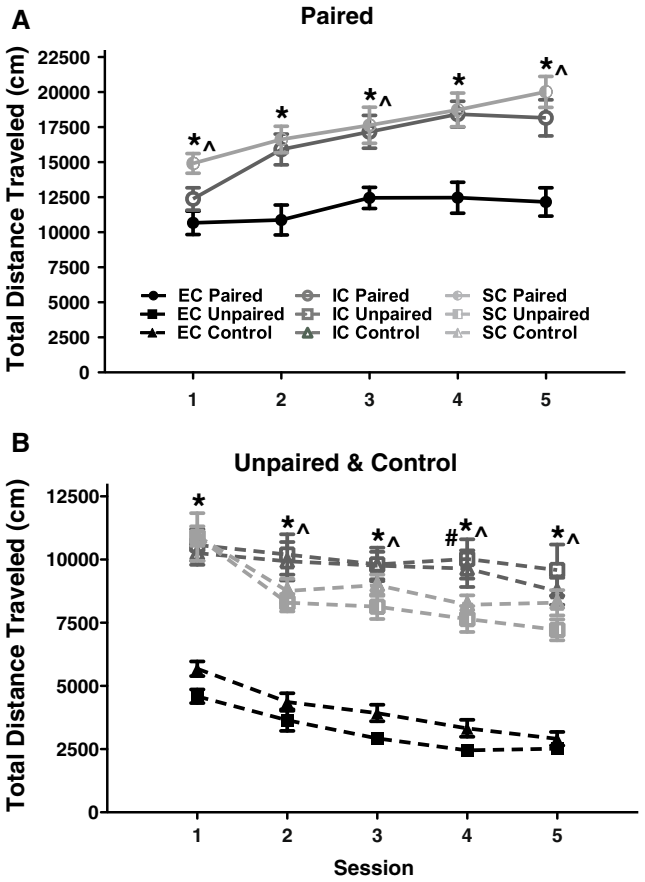

Fig. 3 Total locomotor distance (centimeters) traveled during acquisition in EC, IC, and SC paired (**a**) and unpaired and control (**b**) rats. All paired rats had significantly greater locomotor activity compared to unpaired and control rats. Additionally, all EC treatment groups demonstrated attenuated locomotor activity compared to IC and SC treatment groups. *$p<0.05$ significant difference between EC and IC/SC rats. ^$p<0.05$ significant difference between IC and SC paired or unpaired rats. #$p<0.05$ significant difference between IC and SC control rats

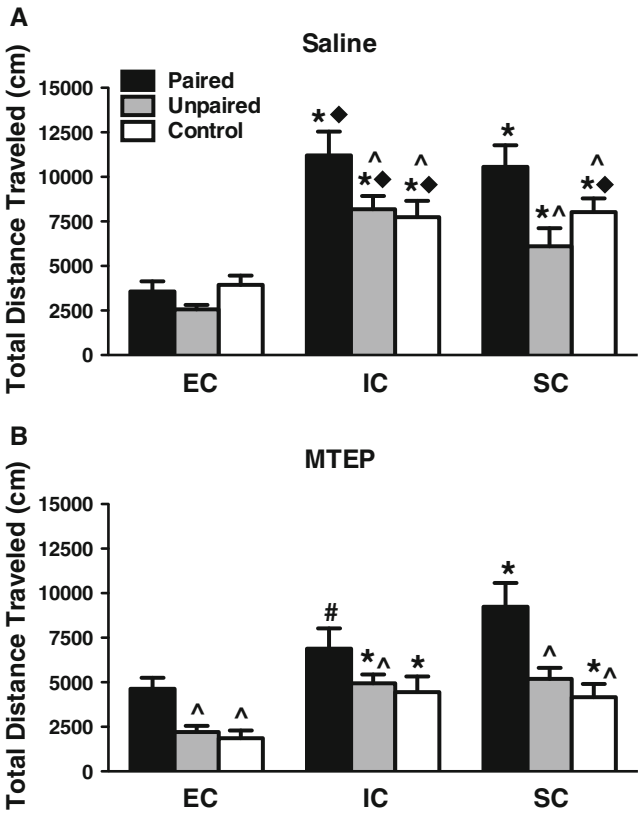

Fig. 4 Total locomotor distance (centimeters) traveled during the conditioned hyperactivity test for saline (**a**) and MTEP (**b**) rats. *$p<0.05$ significant difference between EC and IC/SC rats. #$p<0.05$ significant difference between IC and SC rats. ^$p<0.05$ significant difference between paired and unpaired/control rats within each environmental condition. ♦$p<0.05$ significant difference between MTEP and saline rats

Conditioned hyperactivity test

Prior to the conditioned-hyperactivity test, rats were administered MTEP or saline prior to a saline injection. A 2×3×3 ANOVA revealed a main effect for MTEP treatment [$F(1, 90)=26.63$, $p<0.001$], environmental condition [$F(2, 90)=47.80$, $p<0.001$], and amphetamine treatment [$F(2, 90)=22.66$, $p<0.001$]. Results also showed an environmental condition× MTEP treatment interaction [$F(2, 90)=5.35$, $p<0.01$].

Saline pretreatment

When pretreated with saline, and treated with saline in substitution for amphetamine during the conditioned hyperactivity test, IC and SC rats demonstrated conditioned hyperactivity while EC rats did not (Fig. 4a). Paired IC and SC saline rats had significantly greater locomotor activity than unpaired IC [$F(1, 90)=6.54$, $p<0.05$] and SC [$F(1, 90)=14.15$, $p<0.001$] rats, as well as control IC [$F(1, 90)=8.62$, $p<0.01$] and SC [$F(1, 90)=4.59$, $p<0.05$] saline rats, respectively.

All EC treatment groups displayed decreased locomotor activity compared to IC and SC rats. During the conditioned hyperactivity test, EC paired saline rats had decreased locomotor activity compared to SC [$F(1, 90)=34.87$, $p<0.001$] and IC [$F(1, 90)=41.62$, $p<0.001$] paired saline rats. Additionally, EC unpaired and control saline rats had attenuated locomotor activity compared to SC and IC unpaired [Fs$(1, 90)>8.98$, ps<0.01], as well as SC and IC control [Fs$(1, 90)>10.24$, ps<0.01] saline rats.

MTEP pretreatment

When pretreated with MTEP, all of the environmental conditions displayed conditioned hyperactivity as paired rats had greater locomotor activity than unpaired and control rats (Fig. 4b). This was demonstrated as paired EC, IC, and SC MTEP rats had greater locomotor activity compared to unpaired EC [$F(1, 90)=5.48$, $p<0.05$], IC [$F(1, 90)=4.23$,

$p<0.05$], and SC [$F(1, 90)=18.33$, $p<0.001$] MTEP rats, respectively. Additionally, EC paired and SC paired MTEP rats demonstrated significantly greater locomotor activity compared to control EC [$F(1, 90)=4.20$, $p<0.05$] and SC [$F(1, 90)=11.68$, $p<0.001$] MTEP rats, respectively.

There were also significant differences in locomotor activity between environmental groups pretreated with MTEP, as EC rats had attenuated locomotor activity compared to IC and SC rats. EC paired MTEP rats demonstrated decreased locomotor activity compared to SC [$F(1, 90)=15.10$, $p<0.001$] paired MTEP rats. IC paired MTEP rats had attenuated locomotor activity compared to SC paired MTEP rats [$F(1, 90)=3.96$, $p<0.05$]. In general, EC unpaired and control MTEP rats had decreased locomotor activity compared to SC and IC unpaired and control MTEP rats (Fig. 4b).

MTEP vs. saline pretreatment

Pretreatment with MTEP significantly attenuated locomotor activity primarily in IC rats, but not EC rats compared to saline pretreatment. This was demonstrated as MTEP pretreatment attenuated locomotor activity in paired [$F(1, 90)=13.38$, $p<0.001$], unpaired [$F(1, 90)=9.97$, $p<0.01$], and control [$F(1, 90)=5.54$, $p<0.05$] IC rats compared to pretreated saline rats (Fig. 4a, b). Additionally, MTEP pretreatment attenuated locomotor activity in SC control rats compared to SC saline pretreated control rats [$F(1, 90)=5.74$, $p<0.05$], but had no effect on SC paired and unpaired rats.

Sensitization training

During the sensitization training sessions, rats were administered with amphetamine or saline prior to locomotor sessions. No MTEP was administered during this phase. Results of sensitization training were similar to acquisition results as EC rats displayed attenuated locomotor activity compared to IC and SC rats. Additionally, paired rats within each environmental condition had greater locomotor activity than unpaired and control rats.

A 3×3×5 ANOVA showed a main effect of environmental condition [$F(2, 99)=49.76$, $p<0.001$] and a main effect of amphetamine treatment [$F(2, 99)=198.88$, $p<0.001$]. Analysis also revealed a main effect of session [$F(4, 396)=2.54$, $p<0.05$] and a session×amphetamine treatment interaction [$F(8, 396)=3.51$, $p<0.001$].

Locomotor activity was attenuated in EC compared to IC and SC rats in all treatment conditions. During all five sessions of sensitization training, EC paired rats demonstrated attenuated locomotor activity compared to IC paired [$Fs(1, 396)>85.69$, $ps<0.001$] and SC paired [$Fs(1, 396)>79.54$, $ps<0.001$] rats (Fig. 5a). IC paired rats had attenuated locomotor activity compared to SC paired rats during session 5 [$F(1, 396)=6.09$, $p<0.05$]. Additionally, during all five sessions of sensitization training, EC unpaired and control rats displayed attenuated locomotor activity compared to IC and SC unpaired [$Fs(1, 396)>26.97$, $ps<0.001$] and control [$Fs(1, 396)>42.64$, $p<0.001$] rats (Fig. 5b).

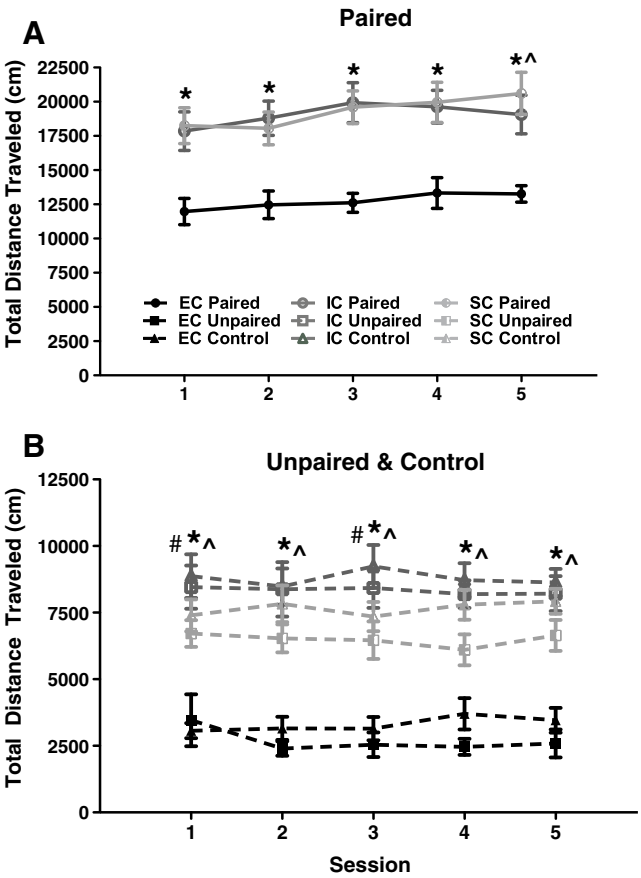

Fig. 5 Total locomotor distance (centimeters) traveled during sensitization training for paired (**a**) and unpaired/control (**b**) rats. All paired rats had significantly greater locomotor activity compared to unpaired and control rats. All EC treatment groups displayed attenuated locomotor activity compared to IC and SC treatment groups. *$p<0.01$ significant difference between EC and IC/SC rats, $p<0.001$. ^$p<0.05$ significant difference between IC and SC paired or unpaired rats. #$p<0.05$ significant difference between IC and SC control rats

Sensitization test

Prior to the sensitization test, rats were administered MTEP or saline 30 min prior to an amphetamine challenge. A 2×3×3 ANOVA revealed a main effect for MTEP treatment [$F(1, 90)=15.10$, $p<0.001$], environmental condition [$F(2, 90)=9.49$, $p<0.001$], and amphetamine treatment [$F(2, 90)=5.60$, $p<0.01$].

Saline pretreatment

When pretreated with saline and treated with amphetamine during the sensitization test, simple effects revealed no effect of treatment. There was a significant effect of environmental

condition when rats were pretreated with saline, as EC control rats displayed attenuated locomotor activity compared to SC control rats [$F(1, 90)=4.91$, $p<0.05$] (Fig. 6a).

MTEP pretreatment

Similar to saline pretreatment, when rats were pretreated with MTEP, none of the rats displayed sensitization, and there was no effect of treatment during the sensitization test. There was a significant effect of MTEP in EC and SC rats, as paired EC and unpaired EC rats had attenuated locomotor activity compared to paired [$F(1, 90)=4.65$, $p<0.05$] and unpaired [$F(1, 90)=5.28$, $p<0.05$] SC rats (Fig. 6b).

MTEP vs. saline pretreatment

Pretreatment with MTEP significantly attenuated locomotor activity in the majority of IC rats, but not EC or SC rats, compared to saline pretreatment. This was demonstrated as MTEP pretreatment attenuated locomotor activity in IC paired [$F(1, 90)=4.11$, $p<0.05$] and IC unpaired [$F(1, 90)=4.65$, $p<0.05$] rats compared to saline pretreatment (Fig. 5a, b).

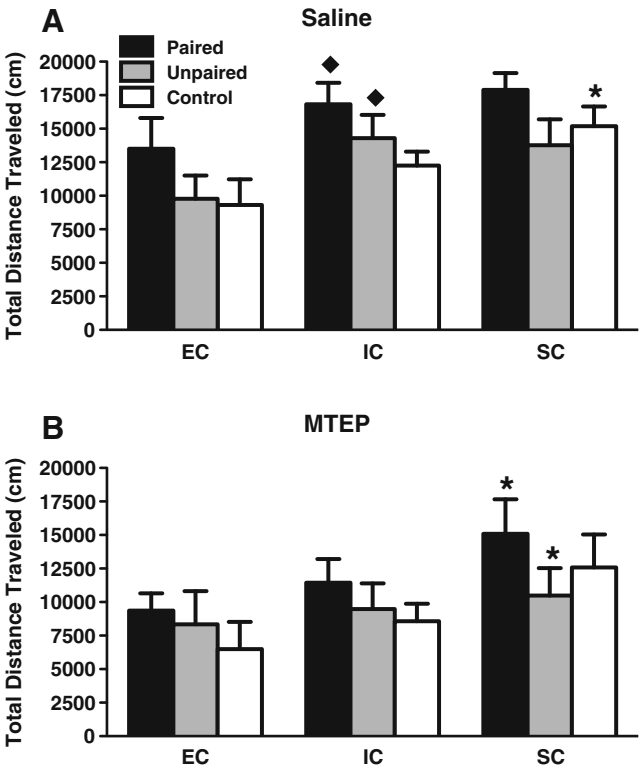

Fig. 6 Total locomotor distance (centimeters) traveled during the sensitization test for saline (**a**) and MTEP (**b**) rats. *$p<0.05$ significant difference between EC and SC rats. ♦$p<0.05$ significant difference between MTEP and saline rats

Discussion

Results of the current study indicate that MTEP attenuates amphetamine-induced hyperactivity following acute, but not repeated injections, and does not alter the expression of conditioned hyperactivity in standard-housed rats. Further, the results support our hypothesis that changes within the glutamatergic system contribute to the ability of environmental enrichment to protect against drug abuse. In the current study, MTEP significantly attenuates acute amphetamine-induced hyperactivity when a low dose of amphetamine is administered in EC and SC, but not IC rats. In contrast, MTEP only attenuates amphetamine-induced hyperactivity in IC rats following repeated amphetamine administration. While MTEP administration attenuated locomotor activity in IC rats, it did not alter expression of conditioned hyperactivity. Interestingly, MTEP administration did alter conditioned hyperactivity in EC rats, as it resulted in the expression of conditioned hyperactivity. Taken together, these findings suggest that glutamatergic changes occur in EC, IC, and SC rats during rearing, which alter the behavioral effects of MTEP.

When rats are repeatedly administered amphetamine during acquisition, results reveal that EC rats have attenuated locomotor activity compared to IC and SC rats. This is consistent with previous research suggesting that EC rats are less sensitive than IC rats to repeated amphetamine administrations (Bardo et al. 1995). Data also revealed that SC paired rats have similar locomotor activity compared to IC paired rats. The results of the current study are consistent with recent findings that show similar psychostimulant-induced locomotor activity in SC and IC rats when administered a moderate to high dose of amphetamine or methylphenidate (Gill et al. 2011; Wooters et al. 2011). In the current study, we hypothesized that administration of MTEP prior to conditioned hyperactivity would attenuate the expression of conditioned hyperactivity in EC paired rats compared to IC and SC paired rats. Interestingly, EC rats did not express conditioned hyperactivity, but MTEP administration resulted in the expression of conditioned hyperactivity in EC rats. MTEP did not enhance or prevent the expression of conditioned hyperactivity in IC and SC rats. While MTEP decreased activity in IC rats during the conditioned hyperactivity test, IC paired rats still expressed conditioned hyperactivity when compared to unpaired rats. Thus, the current study suggests that rearing rats in different environmental conditions alters the pathways involved in Pavlovian conditioning and thus, drug-paired contextual conditioning.

Several studies using the CPP paradigm have demonstrated differences in context-specific learning in differentially reared rats, as enrichment appears to influence conditioned hyperactivity, and thus, one's vulnerability to relapse. Previous research reveals that EC rats demonstrate greater amphet-

amine CPP than IC rats at a low, but not a high dose of amphetamine (Bowling and Bardo 1994). However, a few studies did not observe an effect of differential rearing on psychostimulant CPP immediately following conditioning (Schenk et al. 1986; Solinas et al. 2008). In the current study, we observed conditioned hyperactivity following a 0.3 mg/kg dose of amphetamine in IC, but not EC rats. Additionally, when investigating the role of MTEP administration prior to conditioned hyperactivity, results revealed that MTEP effectively attenuated locomotor activity in all IC treatment groups; however, it did not attenuate locomotor activity in SC paired or unpaired treatment groups. Interestingly, MTEP had the opposite effect in EC rats, as treatment with MTEP resulted in the expression of conditioned hyperactivity. These findings suggests that environmental enrichment may have a protective effect against conditioned hyperactivity, and thus, may protect against Pavlovian conditioned cue-induced relapse when trained using low to moderate doses of psychostimulants. As the current study only observed conditioned hyperactivity in EC rats when MTEP was administered, it suggests that conditioned hyperactivity may rely on mGluR5 function in EC rats.

The current results suggest that MTEP does not attenuate the expression of Pavlovian psychostimulant-paired contextual conditioning in standard-housed rats. While the majority of previous research has focused on the effects of mGluR5 antagonists on the acquisition of Pavlovian conditioning (McGeehan and Olive 2003; Rutten et al. 2010; Veeneman et al. 2011), the current results are consistent with a previous study that did not observe an attenuation of cocaine-CPP expression following MPEP (Herzig and Schmidt 2004). Further, infusion of a group I antagonist, AIDA, into the NAcc does not alter the expression of amphetamine-induced conditioned hyperactivity (Kim et al. 2008). Therefore, while mGluR5 receptors may be critical for the acquisition of psychostimulant contextual conditioning, they do not appear to be critical for the expression of this Pavlovian conditioned response in standard-housed rats.

Results of the acute amphetamine experiment reveal that MTEP attenuates amphetamine-induced hyperactivity in EC and SC rats when a low dose of amphetamine is administered, but there is no effect of MTEP when a moderate dose of amphetamine is administered. These results are consistent with the recent findings that MTEP does not attenuate psychostimulant-induced hyperactivity, following a high dose of cocaine administered acutely (Veeneman et al. 2011). The current experiment and Veeneman et al. (2011) both used the same dose of MTEP (1.0 mg/kg); therefore, it is possible that a higher dose of MTEP is necessary to attenuate acute amphetamine-induced hyperactivity when a higher dose of psychostimulant is administered. The current results contrast with those of Gormley and Rompere (2010) as they observed an attenuation of amphetamine-induced hyperactivity following pretreatment of MPEP, when a moderate dose of amphetamine was used. However, as MPEP has less specificity than MTEP, and as the authors observed an overall attenuation of locomotor activity in the same study, the differences observed may be due to an overall locomotor attenuation that is not specific to mGluR5 function. Interestingly, while MTEP attenuated amphetamine-induced hyperactivity in EC and SC rats when a low dose of amphetamine was administered, it had no effect on hyperactivity in IC rats. The inability of MTEP to attenuate hyperactivity in the IC rats may be due to differences in glutamatergic function between EC and IC rats. IC rats have reduced mGluR function in the PFC (Melendez et al. 2004) and reduced amphetamine-induced glutamate release in the NAcc (Rahman and Bardo 2008).

In contrast to the MTEP-induced attenuation in EC and SC rats when psychostimulant was administered acutely, following repeated psychostimulant administration, MTEP only attenuated psychostimulant-induced locomotor activity in IC paired and unpaired rats. These findings may indicate that rearing specific changes of mGluR5 are differentially influenced by acute versus repeated psychostimulant administration. Interestingly, these findings are consistent with differences in amphetamine-induced locomotor activity of EC and IC rats as EC rats are more sensitive to the rewarding effects of acute amphetamine compared to IC rats. However, following repeated amphetamine administration EC rats exhibit less sensitization than IC rats (Bardo et al. 1995).

In the current study, the absence of an MTEP-induced effect in standard-housed rats during the sensitization test is consistent with previous research, as Dravolina et al. (2006) did not observe an effect of MTEP on cocaine-induced behavioral sensitization. The inability of MTEP to attenuate sensitization may be due to repeated psychostimulant exposure as Hao et al. (2010) demonstrated that mGluR5 expression decreased as rats became cocaine dependent. However, Veeneman et al. (2011) did observe an effect of MTEP on cocaine sensitization with a very high cocaine dose (30 mg/kg, ip).

The results suggest that mGluR5 impacts acute amphetamine-induced hyperactivity to a greater extent than amphetamine-induced sensitization in standard-housed rats. The current results also suggest that mGluR5 is involved in amphetamine-induced locomotor activity, conditioned hyperactivity, and sensitization in differentially reared rats. MTEP attenuated amphetamine-induced hyperactivity in EC and SC rats when amphetamine was administered acutely and hyperactivity in IC rats when amphetamine was administered repeatedly. The effects of MTEP in differentially reared rats support our hypothesis that differential rearing alters glutamatergic pathways, including mGluR5; however, further studies are needed to pinpoint the exact neurobiological mechanisms involved to confirm this hypothesis. These results

have important clinical implications as they suggest that the ability of environmental enrichment to protect against drug abuse may be due to enrichment-induced changes in mGluR5 function. With further research, we may be able to determine how and where mGluR5 function is altered, thus, assisting in the development of better pharmacotherapies to alleviate drug dependence.

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