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The effects of mGluR_{2/3} activation on acute and repeated amphetamine-induced locomotor activity in differentially reared male rats

David L. Arndt¹, Jennifer C. Arnold², and Mary E. Cain¹

¹Kansas State University, Manhattan, KS

²Louisiana State University Health Sciences Center, Shreveport, LA

Abstract

Environmental stimuli play a key role in affecting the likelihood to abuse drugs. Environmental enrichment can reduce that likelihood. Importantly, glutamate contributes to both drug reward and rearing-induced changes in the brain. The current study investigated the effects of the Group II metabotropic glutamate receptor (mGluR_{2/3}) agonist, LY-379268 (0.5, 1.0 mg/kg), on acute and repeated amphetamine-induced locomotor activity in differentially reared male rats. Male Sprague-Dawley rats were randomly assigned to one of three environmental conditions post-weaning: enriched (EC), isolated (IC), or standard (SC), where they reared for 30 days. The effect of LY-379268 on acute amphetamine-induced locomotor activity was assessed. Rats were injected with either LY-379268 (0.5, 1.0 mg/kg) or saline prior to an amphetamine (0.5 mg/kg) or saline challenge injection. Rats were also administered amphetamine (0.5 mg/kg) or saline injections prior to 5 locomotor sessions. Following a rest period of 14–15 days, the effects of repeated amphetamine exposure were evaluated using LY-379268 (0.5; 1.0 mg/kg) or saline injections 30 minutes prior to receiving amphetamine (0.5 mg/kg). Results showed that LY-379268 administration dose-dependently attenuated acute amphetamine-induced locomotor activity, with EC rats generally displaying less attenuation compared to IC or SC rats. After repeated amphetamine administrations, the ability of LY-379268 to attenuate the final expression of amphetamine-induced locomotor activity in differentially reared rats was dose dependent. The differing effect of LY-379268 observed in EC rats suggests enrichment-induced glutamatergic alterations that may protect against sensitivity to psychostimulants.

Keywords

Amphetamine; glutamate; locomotor activity; metabotropic glutamate receptor

Environmental differences are one important factor that can influence both the onset and the continued use of drugs. For example, previous research has shown that differentially rearing

[✉]Corresponding Author: David L. Arndt, Kansas State University, Department of Psychology, 492 Bluemont Hall, Manhattan, KS 66506, david23@ksu.edu.

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rats in enriched (EC), isolated (IC), or standard (SC) environments during adolescence will lead to both neurochemical and behavioral changes in the brain (Rosenzweig et al., 1972; Renner & Rosenzweig, 1987). For example, enrichment enhances glutamate dependent synaptic plasticity as evidenced by the enlargement of synaptic boutons, higher density of dendritic spines, long term potentiation, and other evidence of enhanced synaptic transmission (Artola et al. 2006; Green & Greenough, 1986; Renner & Rosenzweig, 1987; Sharp et al. 1985; van Praag et al. 2000). While EC and IC rats are housed in the two diverse conditions, SC rats can be used to provide a known laboratory standard for comparison (Cain et al., 2012; Gill et al., 2012; Wooters et al, 2011). The neurochemical and behavioral changes induced by these environmental conditions alter the sensitivity to psychostimulant drugs, which can lead to an increased or decreased likelihood for subsequent drug abuse (Bardo & Dwoskin, 2004).

The effects of differential rearing on sensitivity to psychostimulants, such as amphetamine, are dependent on whether the drug is administered acutely or repeatedly (Stairs & Bardo, 2009). The effects of differential rearing on acute amphetamine-induced locomotor activity are inconsistent. One report suggests that EC rats are more sensitive to acute amphetamine (1.0 mg/kg)-induced locomotor activity than IC rats (Bardo et al., 1995). However, recent results suggest that EC rats display a reduced sensitivity to the acute administration of amphetamine across a range of doses (0.3 mg/kg to 1.0 mg/kg) when compared to IC rats (Cain et al., 2012; Gill et al., 2012).

In contrast to the effects of differential rearing on acute amphetamine-induced locomotor activity, the effects of differential rearing on repeated exposure to low doses of amphetamine are more consistent. With repeated amphetamine exposure, EC rats have less amphetamine-induced locomotor activity than IC rats when low unit doses (0.1 and 0.3 mg/kg) are repeatedly administered. However, when administered a moderate (1.0 mg/kg) dose repeatedly, the effects are less consistent. One study observed that EC rats displayed greater amphetamine-induced locomotor activity than IC rats (Bardo et al., 1995) following repeated amphetamine exposure. However, we have recently demonstrated that enrichment attenuates the response to repeated administrations of a moderate dose of amphetamine (Cain et al., 2012). Despite these dose dependent differences, it is clear that differential rearing alters the response to repeated amphetamine administration.

In addition to environmental factors, certain neurotransmitter systems are also accountable for the response to psychostimulants. Previous research indicates that glutamatergic regulation of the mesolimbic reward pathway contributes to both drug reward and rearing-induced changes in the brain (Vanderschuren & Kalivas, 2000; Rahman & Bardo, 2008; Kalivas, 2009). Interestingly, EC rats have greater levels of glutamate in reward regions in the brain following amphetamine than IC rats (Rahman & Bardo, 2008).

Research investigating the role of glutamate receptors in mediating the response to psychostimulants has gained momentum in recent years, with an emphasis on the Group I (mGluR₅) and Group II (mGluR_{2/3}) metabotropic glutamate receptors. While mGluR_{2/3} is localized presynaptically and perisynaptically, reducing transmission at glutamatergic synapses (Anwyl, 1999; Cartmell & Schoepp, 2000; Gass & Olive, 2008), it appears that

mGluR₅ is primarily localized postsynaptically (Romano et al., 1995; Rodrigues et al., 2005; Gormley & Rompre, 2010). Both mGluR_{2/3} and mGluR₅ are involved in the development of sensitivity to psychostimulants (McGeehan & Olive, 2003; Rahman & Bardo, 2008; Schwendt & McGinty, 2007). Group I mGluR₅s play a key role in amphetamine-induced increases in locomotor activity (Gormley & Rompre, 2010; Veeneman et al., 2011). Both the stimulant function of amphetamine and the reinforcing effects of amphetamine are reduced by the mGluR₅ antagonist MTEP (Kumaresan et al., 2009; Martin-Fardon et al., 2009). Interestingly, while Group I and Group II metabotropic receptors contribute to the response to psychostimulants, they also aid in the maintenance of glutamate homeostasis (Kalivas, 2009).

EC and IC rats do not differ in the basal levels of glutamate within the nucleus accumbens (NAcc), but when EC and IC rats are challenged with amphetamine, EC rats have greater levels of glutamate than IC rats (Rahman & Bardo, 2008). This may be due to differential rearing-induced changes in the functioning of both Group I and Group II metabotropic receptors which results in differences in glutamatergic tone. EC rats have increased expression of mGluR₅s in the prefrontal cortex (PFC) compared to IC rats (Melendez et al., 2004). Consistent with these neurochemical observations, administration of MTEP differentially affects amphetamine-induced hyperactivity in EC, IC, and SC rats (Gill et al., 2012). While EC and IC rats do not differ in their basal expression of mGluR_{2/3} in the PFC or striatum, administration of the mGluR_{2/3} antagonist LY-341495 elevates extracellular glutamate in EC and SC rats, but not IC rats (Melendez et al., 2004).

Therefore, to determine if differential rearing-induced changes to Group II metabotropic glutamate receptors alter the behavioral response to amphetamine, the current study investigated the effects of the mGluR_{2/3} agonist, LY-379268, on acute and repeated amphetamine-induced locomotor activity in differentially reared rats. We hypothesized that activation of mGluR_{2/3} via administration of LY-379268 will attenuate amphetamine-induced locomotor activity following both acute and repeated amphetamine administrations compared to saline. We further hypothesized that the effects of mGluR_{2/3} activation would be more pronounced in IC and SC rats compared to EC rats due to the effects of differential rearing on glutamatergic tone.

Materials and methods

Subjects

72 Male Sprague-Dawley rats (Charles River, Portage, MI, USA) were housed in one of three environmental conditions: enriched (EC), isolated (IC), or standard (SC). Rats were then given ad libitum access to food and water throughout the entire experiment. The colony room operated on a 12-hr light-dark cycle and was maintained at 22° C, with humidity ranging from 30–45%. All behavioral tests were conducted during the light portion of the cycle. All procedures conducted and research reported was in accordance with the Institutional Animal Care and Use Committee at Kansas State University, and complied with NIH guidelines (National Research Council, 2011).

Environmental Conditions

Rats arrived in the lab at approximately 21 days of age and were randomly assigned to one of the three environmental conditions. EC rats lived with 11 other cohorts and were housed in a large metal cage ($60 \times 120 \times 45$ cm) that was lined with paper pulp bedding. To maintain novelty and to add to enrichment, EC rats were also handled daily. Fourteen objects (small children's toys and PVC pipe) were regularly rotated and replaced inside the cage. Seven of the objects were changed daily, and all objects were changed twice weekly. IC rats were reared individually and were housed in hanging wire cages ($17 \times 24 \times 20$ cm). IC cages were composed of wire mesh on the front and bottom, with solid sides. The IC rats were not handled throughout the rearing process and were not exposed to novel objects or paper bedding. SC rats were housed in pairs in standard shoebox cages ($20 \times 43 \times 20$ cm). SC rats were exposed to paper bedding, but they did not have any novel objects in their cage and were only handled during the weekly cage change. The inclusion of the SC group of rats was not implemented to control for differences between EC and IC rats, but rather to provide us with a known laboratory standard for comparison. Rats remained in their respective environmental conditions for 30 days and remained in their housing conditions for the entire experimental period.

Apparatus

Locomotor experiments were conducted using 6 identical chambers. The locomotor chambers were $40.64 \times 40.64 \times 40.64$ cm (Coulbourn Instruments, TruScan 2.01) and had clear Plexiglas walls with a stainless steel floor covered with a thin layer of pine-chip bedding. Photobeams were arranged in a 16 (x-axis) photocell array, spaced 2.54 cm apart. Locomotor activity was measured by recording the amount of horizontal movements in centimeters (cm). Throughout every locomotor test session, a 70-db white noise was generated to mask any possible background noise.

Drugs

D-amphetamine (Sigma Aldrich, TX, USA) was dissolved in 0.9% saline (0.5 mg/kg, 1.0 mg/ml) and was administered subcutaneously. LY-379268 (Abcam Biochemicals, MA, USA) was dissolved in 0.9% saline (0.5, 1.0 mg/kg; 1.0 mg/ml) and was administered intraperitoneally.

Behavioral Procedures

Phase 1: Acute Amphetamine—Following the 30-day rearing period, EC, IC, and SC rats were randomly assigned to the amphetamine (0.5 mg/kg) ($n=36$) or saline ($n=36$) treatment group for the duration of the experiment. Rats had two 60-minute locomotor test sessions during the acute phase of the experiment with an intervening rest day in the home cage between the sessions to allow us to make within subjects comparisons to determine the effects of LY-379268. All rats received one treatment of LY-379268 (0.5 or 1.0 mg/kg, i.p.) and saline across the two test sessions. Rats were assigned to the LY-379268 or saline groups in a counterbalanced manner. Thirty minutes following the injection of LY-379268 (0.5 or 1.0 mg/kg, i.p.) or saline, they were transferred to the locomotor room where they were injected with amphetamine or saline immediately prior to being placed individually

into the locomotor chambers. Therefore, each rat received one of the following treatment combinations: LY1.0-AMP and LY1.0-SAL, LY0.5-AMP and LY0.5-SAL, or SAL-AMP and SAL-SAL across the two acute test sessions (Table 1). Different bedding was used in the chamber during each test session to prevent the development of conditioned hyperactivity.

Phase 2: Repeated Amphetamine Administration—Following the acute phase of the experiment, rats rested for approximately one week in their respective environmental conditions to ensure there were no carry-over effects of the LY-379268 exposure during the acute phase of the experiment. Rats then received five, 1-hour locomotor sessions in which the rats received the amphetamine (0.5 mg/kg, s.c.) or saline injections on alternating days. The drug regimen and number of repeated test sessions was based on previous literature (Kim & Vezina, 2002; Kim et al., 2005). Immediately after the drug administration, their locomotor activity was assessed in the chambers. Rats rested in their home cages on alternating days and were not administered amphetamine or saline. During these repeated test days, LY-379268 was not administered.

Repeated Amphetamine Test—After the repeated amphetamine injections, rats rested in their home cages for 14–15 days. After the rest period, rats received an LY-379268 (0.5 or 1.0 mg/kg i.p.) or saline injection thirty minutes prior to a final test. LY-379268 treatment groups were pseudorandomly assigned to ensure that no differences existed in locomotor activity between the rats selected to receive LY-379268 or saline. Immediately prior to being placed in the locomotor chamber for a 60-minute session, all rats received an amphetamine (0.5 mg/kg, s.c.) challenge injection to assess amphetamine-induced locomotor activity.

Data Analyses

Mixed factorial ANOVAs were used to confirm that the order of LY-379268 treatment and amphetamine administration did not alter responding across the acute test sessions. Mixed-factorial ANOVAs were performed to investigate Test Session (LY-379268 versus saline), LY-379268 dose, amphetamine treatment, and environmental condition on total distance traveled (cm) during the acute test session. Test session (LY versus saline) served as a within-subjects factor. For the repeated amphetamine administration tests, mixed factorial ANOVAs were performed, where session served as a within-subjects factor. To test for the expression of repeated amphetamine-induced locomotor activity, a separate between-subjects ANOVA was performed in which LY-379268 dose (0, 0.5 mg/kg, 1.0 mg/kg), amphetamine treatment group, and environmental condition served as between-subjects factors. Post-hoc simple effects were used to probe interactions, and the resulted F-values are reported. Alpha was deemed significant at $p < 0.05$.

Results

Phase 1: Acute Amphetamine

To ensure there was no effect of the order of Test Sessions, a 2 (LY-379268 Order) $\times$ 2 (Test Session) $\times$ 2 (Amphetamine Treatment) $\times$ 3 (Environmental Condition) mixed factorial

analysis of variance (ANOVA) was conducted with order included as a between-subjects factor. Results confirmed no effect of Test Session (LY-379268 versus Saline) order, indicating that the order in which LY-379268 was administered (relative to saline) did not alter responding across sessions. Therefore, the treatment order was not included in subsequent ANOVAs.

Environmental condition, amphetamine treatment, and Test Session (LY-379268 versus Saline) had a significant effect on total distance traveled (cm) during the acute test. A mixed factorial ANOVA revealed a main effect of Environmental condition, $F(2,60)=11.97$, $p<.001$, a main effect of Amphetamine treatment, $F(1,60)=275.07$, $p<.001$, and a main effect of Test Session (LY-379268 versus Saline), $F(1,60)=97.11$, $p<.001$. The ANOVA also revealed several significant two way interactions and a three way interaction between Amphetamine treatment, Test Session (LY-379268 versus Saline), and Environmental condition, $F(2,60)=3.37$, $p<.05$.

LY-379268 Pretreatment Prior to Amphetamine—When compared to saline, both doses of LY-379268 inhibited the expression of acute amphetamine-induced locomotor activity in IC and SC rats, but not EC rats. In IC rats, LY-379268 pretreatment at both 0.5 and 1.0 mg/kg significantly attenuated amphetamine-induced locomotor activity compared to saline pretreatment, $F(1,60)=17.03$, $p<.05$ and $F(1,60)=57.80$, $p<.05$, respectively. In SC rats, LY-379268 pretreatment at both 0.5 and 1.0 mg/kg also significantly attenuated amphetamine-induced locomotor activity compared to saline pretreatment, $F(1,60)=18.02$, $p<.05$ and $F(1,60)=69.65$, $p<.05$ respectively. In SC rats, there was also a significant difference in amphetamine-induced locomotor activity between the high and low dose of LY-379268 pretreatment, $F(1,60)=12.58$, $p<.05$ (Figure 1A).

LY-379268 Pretreatment Prior to Saline—There were no significant effects of LY-379268 in EC-saline rats receiving saline administration. However, the high dose of LY-379268 led to a significant reduction in locomotor activity in both IC and SC-saline rats, $F(1,60)=10.33$, $p<.05$ and $F(1,60)=5.95$, $p<.05$, respectively (Figure 1B).

Saline Pretreatment Prior to Amphetamine or Saline—Differences in amphetamine-induced locomotor activity following saline pretreatment between all three environmental conditions were also examined. In the absence of LY-379268 treatment, EC rats displayed significantly less amphetamine-induced locomotor activity than IC, $F(1,60)=24.61$, $p<.05$, and SC, $F(1,60)=31.76$, $p<.05$, rats (Figure 1A). EC-saline rats had significantly less spontaneous locomotor activity than IC and SC-saline rats, $F(1,60)=12.81$, $p<.05$; $F(1,60)=9.77$, $p<.05$, respectively (Figure 1B).

Phase 2: Repeated Amphetamine Administration

Environmental condition and amphetamine treatment had a significant effect on total distance traveled (Figure 2). Results revealed a significant main effect of Environmental condition, $F(2,66)=36.86$, $p<.001$, and Amphetamine treatment, $F(1,66)=382.86$, $p<.001$. Both of these main effects were qualified by a significant Session $\times$ Amphetamine treatment

interaction, $F(4,264)=4.58, p<.01$, as well as a significant Environmental condition $\times$ Amphetamine treatment interaction, $F(2,66)=8.26, p<.01$.

EC-amphetamine rats displayed significantly less locomotor activity than SC-amphetamine rats during all five sessions, $F's(1,264)=48.75$ to $84.48, p's<.05$. EC rats also displayed significantly less amphetamine-induced locomotor activity than IC rats during all five sessions, $F's(1,264)=15.96$ to $33.58, p's<.05$. In addition, IC rats displayed significantly less amphetamine-induced locomotor activity than SC rats during sessions one through four, $F's(1,264)=5.89$ to $14.37, p's<.05$.

For rats that received saline during the repeated sessions, there was a clear effect of environmental condition on locomotor activity. During each of the 5 sessions, EC-saline rats displayed significantly less locomotor activity than SC-saline rats, $F's(1,264)=29.75$ to $42.21, p's<.05$, and IC-saline rats, $F's(1,264)=84.67$ to $184.17, p's<.05$. In addition, SC-saline displayed significantly less locomotor activity than IC-saline rats during all five sessions, $F's(1,264)=7.55$ to $56.23, p's<.05$.

Phase 3: Repeated Amphetamine Test

Prior to the final test, rats were administered LY-379268 (0.5; 1.0 mg/kg) or saline. Thirty minutes later, all rats received amphetamine (0.5 mg/kg) to determine if the expression of repeated amphetamine-induced locomotor activity could be attenuated by LY-379268. Analyses revealed significant main effects for LY-379268 treatment, $F(1,48)=45.62, p<.001$, and marginal significance for Environmental condition, $F(1,48)=3.09, p=.054$.

LY-379268 Pretreatment Prior to Amphetamine—Compared to saline pretreatment, both doses of LY-379268 significantly attenuated amphetamine-induced locomotor activity in IC and SC rats, but only the high dose of LY-379268 attenuated amphetamine-induced locomotor activity in EC rats, $F(1,54)=31.76, p<.05$. In IC rats, LY-379268 pretreatment at both 0.5 mg/kg and 1.0 mg/kg significantly attenuated amphetamine-induced locomotor activity compared to saline pretreatment, $F(1,54)=6.38, p<.05$ and $F(1,54)=35.78, p<.05$, respectively. In SC rats, LY-379268 pretreatment at both 0.5 mg/kg and 1.0 mg/kg also significantly attenuated amphetamine-induced locomotor activity compared to saline pretreatment, $F(1,54)=8.78, p<.05$ and $F(1,54)=36.58, p<.05$ respectively. There was also a significant attenuation of locomotor behavior in SC-amphetamine rats following the high dose of LY-379268 compared to SC-amphetamine rats given the low dose of LY-379268, $F(1,54)=5.97, p<.05$ (Figure 3A).

LY-379268 Pretreatment Prior to Amphetamine in Previously Saline Treated Rats—The high dose of LY-379268 (1.0 mg/kg) led to a significant reduction in locomotor behavior in EC-saline, IC-saline, and SC-saline rats, $F's(1,54)=10.16$ to $40.01, p's<.05$, treated with saline during training and administered amphetamine during the final test session. Furthermore, the low dose of LY-379268 (0.5 mg/kg) led to a significant reduction in locomotor behavior in only previously IC-saline and SC-saline rats, $F(1,54)=6.61, p<.05$; $F(1,54)=15.29, p<.05$, respectively. There was also a significant attenuation of locomotor behavior in previously IC-saline and SC-saline rats following the high dose of LY-379268

compared to IC-saline rats given the low dose of LY-379268, $F(1,48)=8.60$, $p<.05$; $F(1,54)=5.47$, $p<.05$, respectively (Figure 3B).

Saline Pretreatment Prior to Amphetamine in Previously Saline Treated Rats—

Differences in amphetamine-induced locomotor activity between all three environmental conditions in the absence of LY-379268 were also examined. Results found that previously EC-saline rats significantly differed from previously IC and SC-saline rats, $F(1,54)=6.41$, $p<.05$; $F(1,54)=21.98$, $p<.05$, respectively. Furthermore, previously SC-saline rats significantly differed from previously IC-saline rats, $F(1,54)=4.66$, $p<.05$ (Figure 3B).

Discussion

In general, mGluR_{2/3} activation via LY-379268 decreased acute amphetamine-induced locomotor activity and attenuated the expression of repeated amphetamine-induced locomotor activity. This effect was not only dose-dependent, but varied between environmental conditions. Both doses of LY-379268 attenuated acute amphetamine-induced locomotor activity in IC and SC rats, but not EC rats. The lower dose of LY-379268 (0.5 mg/kg) decreased repeated amphetamine-induced locomotor activity in IC and SC but not EC rats. However, the higher dose of LY-379268 (1.0 mg/kg) attenuated repeated amphetamine-induced locomotor activity in all three environmental conditions. These results are consistent with our hypothesis that the effects of mGluR_{2/3} activation would be more pronounced in IC and SC rats compared to EC rats, and this could be due to enrichment-induced enhancements of glutamatergic tone.

The effects of LY-379268 in SC rats were generally consistent with previous literature (Kim & Vezina, 2002) in that mGluR_{2/3} activation significantly attenuated repeated amphetamine-induced locomotor activity. The current results extend this previous literature by examining an additional dose of LY-379268, and the subsequent effects on the psychostimulating properties of a low amphetamine dose in differentially reared rats. The attenuation of locomotor activity by LY-379268 (0.5 mg/kg) in the acute test was only observed in rats receiving amphetamine, with no effect of LY-379268 treatment evident in rats receiving saline. However, the high dose of LY-379268 (1.0 mg/kg) did attenuate spontaneous locomotor activity in IC and SC rats. Therefore, pretreatment with LY-379268 at 0.5 mg/kg attenuates the psychostimulating effects of amphetamine without altering baseline locomotor activity.

While LY-379268 attenuated the expression of repeated amphetamine-induced locomotor activity in IC and SC rats at the 0.5 mg/kg dose, it inhibited the expression of repeated amphetamine-induced locomotor activity in all three environmental conditions following the 1.0 mg/kg dose. Interestingly, repeated amphetamine administration results in a decrease in mGluR_{2/3} expression in the NAcc, but not in the medial prefrontal cortex (mPFC) (Lu et al., 1997; Lu & Wolf, 1999) in standard-housed rats. Therefore, we predict that differential rearing alters these changes in mGluR_{2/3} expression as a result of repeated amphetamine, and these alterations may account for the differences between the acute and repeated administrations. If the ability of repeated amphetamine to decrease mGluR_{2/3} expression is

blunted in EC rats, we would expect that the lower dose of LY-379268 (0.5 mg/kg) would be less effective in EC rats than the higher LY-379268 (1.0 mg/kg) dose.

Both doses of LY-379268 did not alter acute amphetamine-induced locomotor activity in EC rats, but the high dose (1.0 mg/kg) did attenuate the response to amphetamine during the final test session in EC rats previously administered saline during training. For these EC rats, it was their first exposure to amphetamine and therefore one might expect a similar effect of LY-379268 during both test sessions. However, it is important to note that rats in the saline condition had numerous saline pairings with the locomotor chambers prior to the final amphetamine test. In addition, Group II metabotropic receptors contribute to context and cue-induced reinstatement (Baptista et al., 2004; Bossert et al., 2006; Jin et al., 2010; Lu et al., 2007) and the expression of conditioned hyperactivity (Kim et al., 2008). Therefore, the prior pairings of saline to the locomotor chambers, and the role of mGluR_{2/3} in Pavlovian conditioning, may have also altered the function of mGluR_{2/3} during the test session, and resulted in the ability of the low dose of LY-379268 (0.5 mg/kg) to affect the locomotor response to amphetamine in these EC rats.

Our results suggest that environmental enrichment enhances glutamatergic tone in the presence of amphetamine. This is most likely due to enhanced functioning of mGluR_{2/3} receptors, which is in support of our hypothesis. While basal expression of mGluR_{2/3} receptors in the PFC and striatum do not differ between EC and IC rats, administration of an mGluR_{2/3} antagonist, LY-341495, increases extracellular glutamate in EC and SC rats, but not IC rats (Melendez et al., 2004). These results provide further support for our hypothesis that differential rearing alters glutamatergic tone. The enhanced tone may be due to the presence of mGluR_{2/3} on glial cells (Gass & Olive, 2008). Enrichment increases both the number and density of glial cells (Diamond et al., 1966; Pham et al., 1999). Extracellular glutamate is primarily derived from glial cells (Barbour, 2001; Haydon, 2001; Mazzanti et al., 2001). Therefore, in EC rats, the enhanced amphetamine-induced glutamate release and the evidence of enhanced glutamatergic tone following administration of mGluR_{2/3} agonists and antagonists may be due to the increased number and density of glial cells.

Importantly, glutamatergic afferents from the mPFC synapse on NAcc neurons and augment glutamate release (Bonci et al., 2003; Jay, 2003; Jones & Bonci, 2005; Nicola et al., 2000; Sesack, 2003). These afferents aid in regulation of glutamate release in the synapse and perisynaptic region to maintain glutamate homeostasis. In addition, dysfunction in glutamate homeostasis within the NAcc and mPFC is hypothesized to contribute to drug addiction (Kalivas, 2009). Since differential rearing alters glutamatergic function in multiple brain areas, then it is plausible that IC rats have a dysfunctional reward pathway, which may impact their ability to maintain glutamate homeostasis. Results of the current study may reflect an enhanced glutamatergic homeostatic state in EC rats that may protect against the psychostimulating effects of amphetamine.

In the absence of LY-379268 treatment, the observed effects of differential rearing on amphetamine-induced locomotor activity were consistent with previous literature (Bardo et al., 1995; Cain et al., 2012; Gill, et al., 2012). In general, enrichment decreased the response to amphetamine when compared to isolated and standard rats. This effect was observed

during the acute challenge and the repeated training sessions. Interestingly, during the repeated amphetamine-induced locomotor activity test, there were no differences in the expression of amphetamine-induced locomotor activity in EC, IC, and SC rats. This lack of effect may be due to the fact that we did not observe very robust amphetamine-induced sensitization, which is most likely due to the relatively low training dose of amphetamine. However, it was deemed appropriate to use the low dose of amphetamine because it is the dose in which there are the most noticeable behavioral differences between EC and IC rats (Bardo & Dwoskin, 2004; Cain et al., 2012).

SC rats receiving amphetamine displayed a similar locomotor response as IC rats that received amphetamine. The similarity in the observed behavior between IC and SC rats compared to EC rats is consistent with recent studies (Wooters et al., 2011; Cain et al., 2012; Gill et al., 2012), and suggests that enrichment is attenuating the response to amphetamine, rather than isolation rearing that is increasing the response to amphetamine.

In conclusion, the results of the current study indicate that the mGluR_{2/3} agonist, LY-379268, attenuates both acute amphetamine-induced locomotor activity and repeated amphetamine-induced locomotor activity, and that differential rearing alters this effect. These results extend our previous findings that differential rearing alters the effects of an mGluR₅ antagonist, MTEP (1.0 mg/kg), on amphetamine-induced locomotor activity (Gill et al., 2012) and suggests that differential rearing is also altering the functioning of presynaptic mGluRs. Together, these results suggest that differential rearing is altering both presynaptic and postsynaptic metabotropic receptors and supports our hypothesis that differential rearing alters glutamatergic tone. These changes in glutamatergic tone results in increased (IC rats) or decreased (EC rats) amphetamine-induced locomotor activity. Therefore, enrichment-induced glutamatergic alterations may protect against the sensitivity to psychostimulants.

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All authors contributed in a significant way to the manuscript and all authors have read and approved the final manuscript.

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Figure 1.

A&B. The mean ($\pm$ S.E.M.) total locomotor distance (cm) traveled during the acute amphetamine test session for rats administered amphetamine (A) or saline (B). **A:** Asterisks (*) indicate a significant difference between LY-379268 treatment and saline treatment. Caret sign (^) indicates a significant difference between SC rats administered the low (0.5 mg/kg) and high (1.0 mg/kg) dose of LY-379268. Numerical sign (#) indicates a significant difference between EC and IC/SC rats treated with saline and amphetamine. **B:** Asterisks (*) indicate a significant difference between the high dose of LY-379268 (1.0 mg/kg) and

saline. Numerical sign (#) indicates a significant difference between EC and IC/SC rats treated with saline ($p<.05$).

Figure 2.

The mean ($\pm$ S.E.M.) total locomotor distance (cm) traveled during repeated amphetamine training sessions for rats administered amphetamine or saline. Asterisks (*) indicate a significant difference between EC and IC/SC rats treated with amphetamine or saline. Caret signs (^) indicate a significant difference between IC and SC rats treated with amphetamine. Numerical signs (#) indicate a significant difference between SC and IC rats treated with saline ($p < .05$).

Figure 3.

A&B. The mean ($\pm$ S.E.M.) total locomotor distance (cm) traveled during the final test session during which all rats received amphetamine. **A:** These rats were administered amphetamine throughout training. Asterisks (*) indicate a significant difference between amphetamine rats pretreated with LY-379268 and amphetamine rats pretreated with saline. Caret sign (^) indicates a significant difference between SC-amphetamine rats given the high dose of LY-379268 and SC-amphetamine rats given the low dose of LY-379268. **B:** These rats were administered saline throughout training and amphetamine during the final test

session. Asterisks (*) indicate a significant difference between LY-379268 and saline rats. Caret sign (^) indicates a significant difference between the low (0.5 mg/kg) and high (1.0 mg/kg) dose of LY-379268 in both IC and SC rats. Numerical sign (#) indicates a significant difference between EC and IC/SC rats in the absence of LY-379268 treatment. Ampersand (&) indicates a significant difference between SC and IC rats in the absence of LY-379268 treatment ($p < .05$).

Table 1

Amphetamine (Amp) and Saline treatment groups for enriched (EC), isolated (IC), and standard (SC) rats receiving LY-379268 (0.5; 1.0 mg/kg i.p.) or saline. The n refers to the number of rats from each environmental condition in each treatment condition listed. During the Repeated Amp. Test, the “Previously” refers to the drug condition during the repeated Amp. Administration phase of the experiment.

Environmental Condition (n=24 each)	Acute Test (n=12 each)	Repeated Amp. Administration (n=36 each)	Repeated Amp. Test[*] (n=12 each)
EC, IC, or SC	LY 0.5→Amp	Amp or Saline	Previously Amp→ LY 0.5→Amp
	LY 0.5→Saline		Previously Saline→ LY 0.5→Amp
	LY 1.0→Amp		Previously Amp→ LY 1.0→Amp
	LY 1.0→Saline		Previously Saline→ LY 1.0→Amp
	Saline→Amp		Previously Amp→ Saline→Amp
	Saline→Saline		Previously Saline→ Saline→Amp

^{*} Note: LY treatment counterbalanced from Acute