THE ROLE OF mGluR5 DURING CONDITIONED HYPERACTIVITY AND SENSITIZATION IN DIFFERENTIALLY REARED RATS

by

MARGARET J. GILL

B.A., Luther College, 2005
M.S., Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfuillment of the requirements for the degree

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Abstract

Glutamate contributes to the neurological and behavioral changes that occur during differential rearing, and those that occur during conditioned hyperactivity and sensitization. Metabotropic glutamate receptor 5 (mGluR5) in particular contributes to the psychostimulant reward pathway, plasticity, and differential rearing. The present study examined the role of mGluR5 in conditioning and sensitization in differentially reared rats. Rats were reared in an enriched (EC), impoverished (IC), or social (SC) condition for 30 days, after which they received repeated amphetamine (0.3 mg/kg) or saline injections. Following training, rats received an injection of the mGluR5 antagonist MTEP or saline prior to undergoing conditioned hyperactivity and sensitization tests. Results showed that MTEP attenuated conditioned hyperactivity and sensitization in IC but not EC and SC rats, suggesting that glutamatergic changes occur during differential rearing that alter the effects of MTEP on amphetamine conditioning and sensitization. Additionally, results demonstrated that enrichment rearing has a protective effect against conditioned hyperactivity at low doses of amphetamine.

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Major Professor
Dr. Mary E. Cain

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Glutamate contributes to the changes that occur during differential rearing, and those that occur during conditioned hyperactivity and sensitization. Metabotropic glutamate receptor 5 (mGluR5) in particular contributes to the psychostimulant reward pathway, plasticity, and differential rearing. The present study examined the role of mGluR5 in conditioning and sensitization in differentially reared rats. Rats were reared in an enriched (EC), impoverished (IC), or social (SC) condition for 30 days, after which they received repeated amphetamine (0.3 mg/kg) or saline injections. Following training, rats received an injection of the mGluR5 antagonist MTEP or saline prior to undergoing conditioned hyperactivity and sensitization tests. Results showed that MTEP attenuated conditioned hyperactivity and sensitization in IC but not EC and SC rats, suggesting that glutamatergic changes occur during differential rearing that alter the effects of MTEP on amphetamine conditioning and sensitization. Additionally, results demonstrated that enrichment rearing has a protective effect against conditioned hyperactivity at low doses of amphetamine.

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Enrichment Paradigm

Environmental factors influence drug abuse. However, due to the broad range of environmental factors that influence drug abuse, determining the specific contributing mechanism is difficult. One environmental factor that appears to influence drug abuse during adolescence is differential rearing. Rearing animals in various environments post-weaning allows researchers to investigate how specific neuroanatomical and behavioral changes that occur during rearing influence subsequent drug abuse. Specifically, the enrichment paradigm typically consists of three environmental contexts, an enriched condition (EC), an impoverished condition (IC), and a social condition (SC); though the terms used to define environmental enrichment and the type of environmental enrichment differs between laboratories (Renner & 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 & Dwoskin, 2004; Renner & Rosenzweig, 1987).

Much of the original literature investigating the enrichment paradigm compared EC and IC rearing contexts; however, which social context is used, if any, is variable. The social context often used today is the SC, as it is the standard rearing condition as defined by National Institute of Health (NIH) guidelines (National Research Council, 1996). SC rats are reared in pairs in standard shoebox cages without novel objects. The second type of social context that rats are sometimes reared in is the grouped condition in which several rats are reared in an EC cage without novel objects (Renner & Rosenzweig, 1987). However, in many instances only enriched and impoverished contexts are implemented as rats reared in social contexts do not differ from IC rats (Mirmiran, van den Dungen, & Uylings, 1982). Originally, Welch, Brown,

Welch, and Lin (1974) observed that social housing created neurological differences. However, Rosenzweig, Bennett, Hebert, & Morimoto (1978) later showed that while social housing may contribute to some of the enrichment effects, it is not involved in the neurological characteristics that develop during enrichment rearing. Thus, while the social condition may be important for comparisons with studies using standard rearing practices, it does not appear to be essential when investigating the effects of enrichment rearing.

As the present study contributes to the drug abuse literature as a whole in addition to the enrichment literature, EC, IC, and SC rearing were utilized. These three environmental conditions are based on the work of Rosenzweig, Renner, and Greenough (Greenough, Volkmar, & Juraska, 1973; Renner & Rosenzweig, 1987). Accordingly, EC rats were reared in groups, had novel objects in the cage that were changed daily, and the rats were handled daily. IC rats were reared individually, had no novel objects in the cage, and were not handled during the rearing period. SC rats were housed in pairs, had no novel objects, and they were only handled once a week during the rearing period.

Differential rearing causes several neurological and behavioral changes in EC compared to IC and SC rats. The brains of EC rats are larger than those of IC rats, and these differences in size correlate with changes in the neurons in the brain (Renner & Rosenzweig, 1987). EC rats have a greater number of dendritic spines in the occipital and temporal cortices (Greenough et al., 1973; Volkmar & Greenough, 1972), have a greater number of myelinated axons and fewer unmyelinated axons (Kopcik, Juraska, & Washburne, 1986), and show an increase in neurogenesis (Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999; Segovia, Yague, Garcia-Verdugo, & Mora, 2006).

Researchers have demonstrated that differential rearing causes changes in learning and memory as EC rats are less active than IC rats in an inescapable novel environment (Bardo & Dwoskin, 2004; Bowling, Rowlett, & Bardo, 1993; Lore & Levowitz, 1966). Additionally, EC rats perform better than IC rats in complex tasks, however, EC and IC rats do not differ in performance on simple tasks (Bardo & Dwoskin, 2004; Bardo, Klebaur, Valone, & Deaton, 2001; Domjan, Schorr, & Best, 1977; Renner & Rosenzweig, 1987). EC and IC rats also differ in Pavlovian conditioning. EC rats process contextual conditioning cues faster and are better at discriminating between conditioned stimuli than SC rats, however, EC and IC rats display similar levels of freezing when presented with a Pavlovian fear conditioned stimulus (Barbelivien et al., 2006; Duffy, Craddock, Abel, & Nguyen, 2001; Woodcock & Richardson, 2000).

In the current study we reared rats for 30 days, as previous research suggests that both neuroanatomical and behavioral changes occur following 30 days of rearing. Previous research has shown that neuroanatomical changes begin to occur after four days of rearing in differential environments (Rosenzweig & Bennett, 1978). Zhu, Apparsundaram, Bardo, and Dwoskin (2005) demonstrated that EC and IC rats differed in dopamine transporter (DAT) surface expression following 30 days of rearing. Meanwhile, behavioral changes occur in rats between 16 and 45 days of age, and are not altered after 45 days of age (Einon & Morgan, 1977). Thus, behavioral differences in EC and IC rats should be apparent following 30 days of differential rearing.

Differential Rearing and Psychostimulants.

As mentioned above, neurological and behavioral changes occur when rats are differentially reared, and interestingly, these differences appear to be exacerbated following

psychostimulant conditioning. Differentially reared rats display differences in conditioned hyperactivity following amphetamine training. Conditioned hyperactivity occurs when rats in a drug free state are exposed to a previously drug-paired environment. This drug-environment pairing leads to a context specific increase in locomotor activity compared to activity when rats are not in a drug paired environment (Ahmed, Stinus, & Cador, 1998; Barr et al., 1983; Gold, Swerdlow, & Koob, 1988; Pickens & Crowder, 1967). As the drug and neutral environment are repeatedly paired, Pavlovian conditioning occurs (Gold et al., 1988). These context specific cues are often responsible for drug relapse as re-exposure to contextual cues elicits drug paired conditioned hyperactivity. The effects of differential rearing on conditioned hyperactivity appear to be dose dependent. Research in our laboratory has shown that both EC and IC rats display conditioned hyperactivity when trained using a 0.3 mg/kg dose of amphetamine (Neises, Pittenger, Gill, & Cain, 2006). Bowling and Bardo (1994) observed differences in conditioning between EC and IC rats following a low dose (0.5 mg/kg) of amphetamine using a conditioned place preference paradigm, as EC rats spent significantly more time in the previously drug paired compartment than IC rats. Results from our laboratory revealed conditioned hyperactivity in EC but not IC rats following a 1.0 mg/kg dose of amphetamine, (Neises et al., 2006) however, Bowling and Bardo (1994) found that EC and IC rats did not differ in conditioned place preference when trained using a 2.0 mg/kg dose of amphetamine. These results suggest that differences in conditioning of EC and IC rats are only apparent at low to moderate doses of amphetamine, and that these differences are attenuated at high doses.

In addition to behavioral differences during conditioning, EC and IC rats also differ in behavior following amphetamine administration. Similar to conditioned hyperactivity,

differences between EC and IC rats appear to be dose-dependent. Following acute amphetamine exposure, EC rats show a dose-dependent increase in amphetamine-induced hyperactivity compared to IC rats, as EC rats display greater amphetamine-induced hyperactivity than IC rats following 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 & Bardo, 1994)). EC and IC rats also differ in amphetamine self-administration, as there are no differences in the amount of amphetamine EC and IC rats self-administer at high doses, but at low doses EC rats self-administer less amphetamine than IC rats (Bardo et al., 2001).

Differential rearing also alters behavior following psychostimulant sensitization. Sensitization is the enhancement of psychomotor activity following repeated psychostimulant administration (Stewart & Badiani, 1993; Vanderschuren & Kalivas, 2000). This sensitization effect is enhanced after a 1-4 week rest following repeated amphetamine administrations (Hitzemann, Tseng, Hitzemann, Sampath-Khanna, & Loh, 1977; Hooks, Duffy, Striplin, & Kalivas, 1994; Kalivas & Duffy, 1989, 1993; Kolta, Shreve, De Souza, & Uretsky, 1985; Paulson, Camp, & Robinson, 1991; Uslaner, Crombag, Ferguson, & Robinson, 2003). Studies have shown that EC rats are less sensitive than IC rats to amphetamine-induced sensitization (Bardo et al., 1995; Bardo et al., 2001). Consistent with findings following repeated psychostimulant administration and conditioned hyperactivity, the effects of differential rearing on psychostimulant-induced sensitization are dose dependent. 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).

Role of Dopamine in Drug Reward

The mesolimbic dopamine (DA) pathway is the primary pathway mediating the effects of psychostimulant use. The ventral tegmental area (VTA), nucleus accumbens (NAcc), prefrontal cortex (PFC), amygdala, and the connection between the ventral tegmental area and the basal forbrain are the primary components of the mesolimbic DA pathway (Koob, 1999). Research suggests that the differential effects of amphetamine in EC and IC rats are mediated by DA function, as the mesolimbic DA pathway is responsible for the motivational and rewarding effects of psychostimulants (Bardo et al., 1995; Koob, 1999); and is activated following exposure to a novel environment (Bardo & Dwoskin, 2004; Koob, 1999). Dopamine mediated behaviors are altered differentially in EC and IC rats, as EC and IC rats exhibit differing levels of locomotor activity following administration of a dopamine transporter (DAT) inhibitor (Zhu, Green, Bardo, & Dwoskin, 2004). EC and IC rats also differ in the expression of DA receptors in the PFC (Del Arco et al., 2007), and EC rats have greater levels of DA in the cortex (Riege & Morimoto, 1970). However, there are no EC and IC differences in levels of DA in the NAcc following amphetamine administration (Bardo et al., 1995). Interestingly, EC and IC rats do appear to differ in glutamate levels in the NAcc following amphetamine (Rahman & Bardo, 2008).

Role of Glutamate in Drug Reward

The mesolimbic DA system is responsible for the rewarding effects of psychostimulants, and it is hypothesized that activation of the mesolimbic DA system induces glutamate transmission in a feedback loop which projects from the PFC and amygdala to the VTA and NAcc (Ghitza, Fabbricatore, Prokopenko, & West, 2004; Vanderschuren & Kalivas, 2000). The role of

glutamate in drug reward has been demonstrated as amphetamine administration causes increases in extracellular glutamate in the striatum (Del Arco, González-Mora, Armas, & Mora, 1999; Reid & Berger, 1996; Xue, Ng, Li, & Wolf, 1996). Use of *N*-methyl-$_D$-aspartate (NMDA) antagonists also reveal a correlation between glutamate and drug reward as MK-801, a NMDA antagonist, blocks increases of glutamate following amphetamine (Wolf & Xue, 1999). Glutamate is also involved in psychostimulant sensitization as MK-801 inhibits both sensitization and sensitization-dependent cellular neuroadaptations (Kalivas & Alesdatter, 1993). In addition to the role glutamate plays in drug reward, it appears that it may be involved in the synaptic changes caused by differential rearing, as glutamate influences both synaptic transmission and plasticity.

Contribution of Glutamate To Differential Rearing

Because the neurological changes that occur during differential rearing are associated with synaptic plasticity (Altschuler, 1979a; Duffy et al., 2001; Green & Greenough, 1986b), researchers hypothesize that these changes may be modulated by glutamate (Giorgetti, Hotsenpiller, Ward, Teppen, & Wolf, 2001; Melendez, Gregory, Bardo, & Kalivas, 2004; Wolf, 1998; Wolf & Xue, 1999). Though little research has examined glutamate levels and drug reward following differential rearing, glutamate contributes to both drug reward and rearing-induced changes in the brain. Segovia, Yague, Garcia-Verdugo, and Mora (2006) found that EC rats have greater levels of glutamate in the hippocampus compared to IC rats. EC rats also have significantly greater levels of mGluR5 dimers in the PFC compared to IC rats, though there are no differences in mGluR5 monomers (Melendez et al., 2004). Additionally, EC rats have reduced NMDA receptors compared to IC rats, but there are no differences in AMPA receptors

in the NAcc (Wood, Buse, Wellman, & Rebec, 2005). There are also glutamatergic differences between EC and IC rats following amphetamine administration. Rahmen and Bardo (2008) demonstrated that EC rats have greater levels of glutamate compared to IC rats in the NAcc following amphetamine, while glutamate levels do not differ between rearing groups following saline. This suggests that rearing-induced changes to the glutamatergic system in the NAcc are kindled by exposure to psychostimulants. Though research has revealed that glutamate contributes to neurological differences in differentially reared rats and differential responses following amphetamine administration, researchers have not yet pinpointed what types of glutamate receptors are involved.

Role of mGluR5 in Conditioning and Sensitization

MGluR5 is a viable candidate for contributing to differences in drug use between EC and IC rats due to its involvement in the psychostimulant reward pathway, plasticity, and differential rearing (McGeehan & Olive, 2003; Rahman & Bardo, 2008; Schwendt & McGinty, 2007; van Praag, Kempermann, & Gage, 2001). Both the stimulant function of amphetamine conditioning, and the reinforcing effects of amphetamine are reduced by mGluR5 antagonists.

Conditioning.

While the role of mGluR5 in conditioned hyperactivity has not been investigated, glutamate may be involved in conditioned hyperactivity as MK-801 blocks amphetamine conditioned place preference (CPP) completely when administered prior to the session (Kelley, Anderson, & Itzhak, 2007; Tzschentke & Schmidt, 1997), and cocaine CPP is blocked when glutamate antagonists are injected into the VTA (Harris & Aston-Jones, 2003). Specifically, mGluR5 is hypothesized to contribute to Pavlovian conditioning as the mGluR5 antagonists 3-

((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP) (Kumaresan, et al., 2009; Martin-Fardon, Baptista, Dayas, & Weiss, 2009) and 2-Methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP) (Bäckström & Hyytiä, 2007) both attenuate cue-induced reinstatement (Martin-Fardon et al., 2009). Additionally, researchers have shown that mGluR5 contributes to Pavlovian fear conditioning as MPEP administration into the amygdala attenuated acquisition but not expression or consolidation of contextual fear conditioning (Rodrigues, Bauer, Farb, Schafe, & LeDoux, 2002). The role of mGluR5 in the basolateral amygdala (BLA) was observed during conditioned taste aversion as MTEP infusion into the BLA enhanced conditioned taste aversion (Simonyi, Serfozo, Parker, Ramsey, & Schachtman, 2009). Additional research suggests that mGluR5 is not only involved in Pavlovian conditioning, but specifically the learning process responsible for the association of the conditioned stimulus. O'Connor, Crombag, Mead, and Stephens (2010) investigated the role of mGluR5 in Pavlovian conditioning by administering either MTEP or saline throughout acquisition, and then again prior to a conditioned reinforcement session. Researchers found that mGluR5 is essential for the learning process in which the conditioned stimulus is associated with an incentive, and produces the conditioned response. Though interestingly, mGluR5 does not appear to be essential for expression of the conditioned response after learning occurs. While there is little research investigating the role of mGluR5 in Pavlovian conditioning, mGluR5 most likely contributes to other forms of Pavlovian conditioning such as contextual drug conditioning.

Sensitization.

In contrast with conditioned hyperactivity, much more research has been conducted on the role of mGluR5 in drug sensitization. A reduction in psychostimulant-induced hyperactivity

occurs when mice are administered MPEP (Melendez et al., 2004), however, locomotor activity is not affected when MPEP is administered acutely without psychostimulants (Ossowska, Konieczny, Wolfarth, Wierońska, & Pilc, 2001; Spooren, Gasparini, Bergmann, & Kuhn, 2000). MTEP attenuates methamphetamine-induced reinstatement (Gass, Osborne, Watson, Brown, & Olive, 2009), as well as methamphetamine (Gass et al., 2009) and cocaine (Martin-Fardon et al., 2009) self-administration in a dose dependent manner. MTEP does not attenuate locomotor activity or lever pressing for food (Gass et al., 2009); thus, MTEP is not merely attenuating locomotor activity. Interestingly, Dravolina, Danysz, and Bespalov (2006) did not observe an effect of MTEP on cocaine-induced behavioral sensitization, however, rats were not rested for the standard 1-4 weeks following training and the doses of MTEP administered may have been too high. Previous research has shown an increase in behavioral sensitization following a 1-4 week rest period (Hitzemann et al., 1977; Hooks et al., 1994; Kalivas & Duffy, 1989, 1993; Kolta et al., 1985; Paulson et al., 1991; Uslaner et al., 2003). Preliminary results in our laboratory suggest that the ability of MTEP to decrease sensitization is dose dependent. 1.0 and 3.0 mg/kg MTEP attenuated amphetamine sensitization, but a 0.3 or 0.7mg/kg dose of MTEP did not. However, the 3.0 mg/kg dose of MTEP also attenuated locomotor activity in rats that received saline, thus, the current study used a 1.0 mg/kg dose of MTEP. Dravolina et al. (2006) administered 2.5, 5, and 10 mg/kg of MTEP following a moderate dose of cocaine, thus, using much higher doses than what was found to be optimal in our laboratory. These studies suggest that mGluR5 contributes to maintaining psychostimulant self-administration as well as psychostimulant-induced behavioral effects, while the effects of sensitization using an optimal MTEP dose are unknown.

The involvement of mGluR5 in synaptic plasticity has been evidenced during psychostimulant-induced sensitization. During sensitization, levels of mGluR5 are reduced in the striatum 3 hrs after amphetamine treatment compared to baseline saline treatment (Mao & Wang, 2001). However, another study found increased mGluR5 mRNA levels in the NAcc shell and dorsolateral striatum following cocaine sensitization (Ghasemzadeh, Nelson, Lu, & Kalivas, 1999). Although glutamate levels appear to vary depending upon the area of the brain, nevertheless, these findings suggest that changes in synaptic plasticity occur during sensitization, which may permanently alter glutamate receptors.

Specific mGluR5 Antagonists

The current study used the mGluR5 antagonist MTEP as it is more potent and specific than MPEP. While initial studies suggest that MPEP did not influence other mGlu or NMDA receptors (Gasparini et al., 1999), more recent work reveals that MPEP acts on NMDA receptors (Cosford et al., 2003) and modulates mGluR4 receptors (Mathiesen, Svendsen, Bruner-Osborne, Thomsen, & Ramirez, 2003). MTEP appears to have a greater specificity for mGluR5 receptors (Cosford et al., 2003).

In the current study we investigated the effect of mGluR5 antagonism on the expression of conditioned hyperactivity and sensitization in differentially reared rats. We hypothesized that administration of MTEP prior to conditioned hyperactivity would attenuate conditioned hyperactivity in EC paired rats compared to IC and SC paired rats, while having no effect on unpaired and control rats. It was also hypothesized that MTEP pretreatment would attenuate conditioned hyperactivity compared to paired rats that were pretreated with saline. We hypothesized that MTEP administration prior to sensitization would attenuate locomotor

activity significantly more in EC compared to IC and SC paired rats. Additionally, we predicted that MTEP would decrease sensitization in paired rats pretreated with MTEP compared to saline paired rats, and have no effect on unpaired or control rats. Our hypotheses were based on findings that EC rats display attenuated sensitization compared to IC rats following a low to moderate dose of amphetamine (Bardo et al., 1995; Smith, Neill, & Costall, 1997). This study contributes to the literature as no research has been conducted to date investigating the role of mGluR5 in amphetamine-induced conditioned hyperactivity and sensitization for standard or differentially reared rats.

Method

Subjects

Male Sprague Dawley rats were obtained from Charles River (Portage, MI, USA), and housed in one of three environments described below. The colony was on a 12-hr light-dark cycle with lights on from 0700 to 1900 hrs. The colony was maintained at 22° C and humidity ranged from 30-45%. Rats had ad libitium access to food and water. 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 10-12 in large metal cages (60 x 120 x 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 x 24 x 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 x 43 x 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 cm x 40.64 cm x 40.64 cm (Coulbourn Instruments, TruScan 2.01) and had clear plexiglass walls and a stainless steel floor covered with pine-chip bedding. Locomotor activity was measured by recording photobeam interruptions. Photobeams were arranged in a 16 (X-axis) photocell array, spaced 2.54 cm apart (center to center). Throughout the session a 70-db white noise was generated to mask background noise.

Drugs

D-amphetamine was dissolved in 0.9% saline (0.3 mg/kg, 1.0 mg/mL) and injected subcutaneously. MTEP was dissolved in 0.9% saline (1.0 mg/kg, 1.0 mg/mL) and injected intraperitoneally. Both d-amphetamine and MTEP were obtained from Sigma Aldrich (Dallas, TX, USA).

Statistical Analyses

A power analysis was performed to determine the number of animals needed in the studies (G*POWER; (Faul, Erdfelder, Lang, & Buchner, 2007). The alpha level was set at .05, the

power level was set at 0.80, and was estimated that using 108 animals would produce a large effect size (f = 0.5).

The total distance traveled (cm) during each training phase was analyzed using a 3 X 3 X 5 mixed subjects analysis of variance (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. In addition, responding during each 1-hr test session was analyzed across six 10-minute bins using a mixed subjects ANOVA. As EC, IC, and SC rats had different baselines during acquisition and sensitization training, the total distance traveled (cm) during the conditioned hyperactivity test and sensitization test were standardized using z-scores. Z-score transformations were performed for each individual rat as this accounts for differences in baseline means and variability among groups. Two separate between subjects ANOVAs were then performed using the z-scores. Multiple comparisons with a Bonferroni correction were used to probe any significant interactions. Alpha was deemed significant at $p<.05$.

Procedures

Acquisition.

Following 30 days of rearing, rats (n=108) were assigned to either paired, unpaired, or control groups (n=36 per group) in a counterbalanced manner. Experiments were performed in 3 separate groups, each group consisting of rats from each rearing and treatment condition.

Rats underwent a 1-hr locomotor session for 5 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.

Conditioned hyperactivity test.

After acquisition rats underwent a conditioned hyperactivity test. Rats were administered an MTEP (1 mg/kg, i.p.) or saline injection (Table 1). Thirty minutes later (Palmatier, Liu, Donny, Caggiula, & Sved, 2008) all rats received a saline challenge injection immediately prior to being placed in the locomotor chamber for a 1-hr session.

Sensitization training.

Rats received 5 additional training sessions in the locomotor chambers. Procedures were identical to those during acquisition. Following sensitization training, rats rested in their home cages for 14 days.

Sensitization.

After the 2-week rest period, rats received an MTEP (1 mg/kg, i.p.) or saline injection (Table 1). Thirty minutes later (Palmatier et al., 2008) all rats received an amphetamine (0.3 mg/kg, s.c.) challenge injection immediately prior to being placed in the locomotor chamber. In the sensitization test rats were placed in the locomotor chamber for a 1-hr session.

Results

Acquisition

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 repeated measures ANOVA showed a main effect of environmental condition, $F(2,99)=84.93$, $p<.001$, and a main effect of amphetamine treatment, $F(2,99)=169.99$, $p<.001$. Analysis also revealed a session X environmental condition, $F(8,396)=4.20$, $p<.001$, and a session X amphetamine treatment interaction, $F(8,396)=28.76$, $p<.001$.

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, $Fs(1,396)>6.36$, $ps<.05$, and SC paired, $Fs(1,396)>39.17$, $ps<.001$, rats (Figure 1A). Results revealed that IC paired rats had attenuated locomotor activity compared to SC paired rats during sessions 1, 3, and 5, $Fs(1,396)>6.13$, $ps<.05$. In rats administered saline immediately prior to the session, EC rats tended to have less locomotor activity than IC and SC rats (Figure 1B). Locomotor activity was attenuated in EC unpaired rats compared to IC, $Fs(1,396)>78.03$, $ps<.001$, and SC unpaired, $Fs(1,396)>47.06$, $ps<.001$, rats, as well as EC control rats compared to IC, $Fs(1,396)>45.62$, $ps<.001$, and SC control, $Fs(1,396)>42.13$, $ps<.001$, rats. During sessions 2, 3, 4, and 5, SC unpaired rats demonstrated attenuated locomotor activity compared to IC unpaired rats, $Fs(1,396)>6.13$, $p<.05$. During session 4 SC control rats had attenuated locomotor activity compared to IC control rats, $F(1,396)=4.43$, $p<.05$.

During all 5 sessions of acquisition there was a significant effect of treatment as paired rats within each environmental condition demonstrated greater locomotor activity than unpaired and control rats (Figure 2). During each session, paired EC, IC, and SC rats had greater locomotor activity than unpaired EC, $Fs(1,396)>80.58$, $ps<.001$, IC, $Fs(1,396)>7.10$, $ps<.01$, and SC rats, $Fs(1,396)>35.24$, $ps<.001$, as well as control EC, $Fs(1,396)>54.31$, $ps<.001$, IC, $Fs(1,396)>9.84$, $ps<.01$, and SC $Fs(1,396)>34.53$, $ps<.001$, rats.

MTEP treatments did not begin until conditioned hyperactivity. However, we compared locomotor activity during session 5 in rats that would be assigned to MTEP or saline conditions to ensure there were no baseline differences. There was no main effect of MTEP assignment, $F(1,90)=0.20$, $p=.652$, thus, the observed effects of MTEP on conditioned hyperactivity were not due to baseline differences (Figure 3).

Conditioned Hyperactivity Test

A 2 x 3 x 3 univariate ANOVA revealed a main effect for MTEP treatment, $F(1,90)=26.63$, $p<.001$, environmental condition, $F(2,90)=47.80$, $p<.001$, and amphetamine treatment, $F(2,90)=22.66$, $p<.001$. Results also showed an environmental group X MTEP treatment interaction, $F(2,90)=5.35$, $p<.01$.

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. Analyses revealed a significant effect of treatment group in IC and SC, but not EC rats (Figure 4A). Paired IC and SC saline rats had significantly greater

locomotor activity than unpaired IC, $F(1,90)=6.54$, $p<.05$, and SC, $F(1,90)=14.15$, $p<.001$, rats, as well as control IC, $F(1,90)=8.62$, $p<.01$, and SC, $F(1,90)=4.59$, $p<.05$, saline rats.

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, $F(1,90)=34.87$, $p<.001$, and IC, $F(1,90)=41.62$, $p<.001$, paired saline rats. Additionally, EC unpaired and control saline rats had attenuated locomotor activity compared to SC and IC unpaired, $Fs(1,90)>8.98$, $ps<.01$, as well as SC and IC control, $Fs(1,90)>10.24$, $ps<.01$, 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. (Figure 4B). This was demonstrated as paired EC, IC, and SC MTEP rats had greater locomotor activity compared to unpaired EC, $F(1,90)=5.48$, $p<.05$, IC, $F(1,90)=4.23$, $p<.05$, and SC, $F(1,90)=18.33$, $p<.001$, MTEP rats, respectively. Additionally, EC paired and SC paired MTEP rats demonstrated significantly greater locomotor activity compared to control EC, $F(1,90)=4.20$, $p<.05$, and SC, $F(1,90)=11.68$, $p<.001$, MTEP rats, respectively.

There were also significant locomotor differences 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, $F(1,90)=15.10$, $p<.001$, paired MTEP rats. IC paired MTEP rats had attenuated locomotor activity compared to SC paired MTEP rats, $F(1,90)=3.96$, $p<.05$. EC unpaired rats had decreased locomotor activity compared to IC unpaired MTEP rats, $F(1,90)=4.75$, $p<.05$. Finally, EC control

MTEP rats demonstrated attenuated locomotor activity compared to SC and IC control MTEP rats, *Fs*(1,90)>5.34, *ps*<.05.

Comparing saline and MTEP 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, $F(1,90)=13.38$, $p<.001$, unpaired, $F(1,90)=9.97$, $p<.01$, and control, $F(1,90)=5.54$, $p<.05$, IC rats compared to pretreated saline rats. Additionally, MTEP pretreatment attenuated locomotor activity in SC control rats compared to pretreated saline control rats, $F(1,90)=5.74$, $p<.05$, but had no effect on paired and unpaired rats.

Z-score standardization.

Because there were baseline differences in EC, IC, and SC rats during acquisition, locomotor activity during the conditioned hyperactivity test was standardized (Figures 5A and 5B). Results revealed a main effect for environmental condition, $F(2,90)=47.80$, $p<.001$, a main effect for amphetamine treatment, $F(2,90)=22.66$, $p<.001$, and a main effect for MTEP treatment, $F(1,90)=26.63$, $p<.001$. Additionally, there was a environmental condition X MTEP treatment interaction, $F(2,90)=5.35$, $p<.01$.

Conditioned hyperactivity test timecourse.

To confirm that MTEP was effective during the entire session, MTEP and saline treatment groups were compared during each 10 minute bin throughout the 60 minute conditioned hyperactivity test. Overall, results did not reveal changes in MTEP's effectiveness during the majority of the 60 minute session, though there was only one MTEP and saline

difference during the last bin of the conditioned hyperactivity test (Table 2). Results revealed only two MTEP and saline differences in EC rats throughout the 60 minute session, as MTEP attenuated locomotor activity in unpaired rats during Bin 1, and paired rats during Bin 3 (Figure 6A). MTEP was shown to attenuate locomotor activity in paired, unpaired, and control IC rats during the conditioned hyperactivity test compared to saline rats (Figure 6B). MTEP attenuated locomotor activity in paired, unpaired, and control SC rats during the first 10 min bin; however, it did not significantly attenuate locomotor activity again in SC rats until the 4^{th} and 5^{th} bins of the conditioned hyperactivity test (Figure 6C).

Sensitization Training

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 repeated measures ANOVA showed a main effect of environmental condition, $F(2,99)=49.76$, $p<.001$, and a main effect of amphetamine treatment, $F(2,99)=198.88$, $p<.001$. Analysis also revealed a main effect of session, $F(4,396)=2.54$, $p<.04$, and a session X amphetamine treatment interaction, $F(8,396)=3.51$, $p<.001$.

Locomotor activity was attenuated in EC compared to IC and SC rats in all treatment conditions. During all 5 sessions of sensitization training EC paired rats demonstrated attenuated locomotor activity compared to IC paired, $Fs(1,396)>85.69$, $ps<.001$, and SC paired, $Fs(1,396)>79.54$, $ps<.001$, rats (Figure 7A). IC paired rats had attenuated locomotor activity compared to SC paired rats during session 5, $F(1,396)=6.09$, $p<.05$. Additionally, during all 5 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*<.001, and control, *Fs*(1,396)>42.64, *p*<.001, rats (Figure 7B). SC unpaired rats demonstrated attenuated locomotor activity compared to IC unpaired rats during sessions 1 through 5, and SC control rats demonstrated attenuated locomotor activity compared to IC control rats during sessions 1 and 3.

Results revealed a significant effect of treatment during all 5 sessions of sensitization training as paired rats within each environmental condition demonstrated greater locomotor activity than unpaired and control rats (Figure 8). During each session, paired EC, IC, and SC rats displayed greater locomotor activity compared to unpaired EC, *Fs*(1,396)>184.72, *ps*<.001, IC, *Fs*(1,396)>224.81, *ps*<.001, and SC rats, *Fs*(1,396)>338.02, *ps*<.001, as well as control EC, *Fs*(1,396)>202.05, *ps*<.001, IC, *Fs*(1,396)>205.53, *ps*<.001, and SC, *Fs*(1,396)>266.09, *ps*<.001, rats. Additionally, EC unpaired rats demonstrated attenuated locomotor activity compared to EC control rats during sessions 4, *F*(1,396)=3.92, *p*<.05, and 5, *F*(1,396)=497.27, *p*<.001. Differences in locomotor activity were also observed between SC unpaired and control rats, as SC unpaired rats had attenuated locomotor activity compared to SC control rats during sessions 2, *F*(1,396)=4.30, *p*<.05, 4, *F*(1,396)=7.27, *p*<.01, and 5, *F*(1,396)=4.18, *p*<.05.

Sensitization Test

A 2 x 3 x 3 univariate ANOVA revealed a main effect for MTEP treatment, *F*(1,90)=15.10, *p*<.001, environmental condition, *F*(2,90)=9.49, *p*<.001, and amphetamine treatment, *F*(2,90)=5.60, *p*<.01.

Saline pretreatment.

When pretreated with saline and treated with amphetamine during the sensitization test, simple effects revealed no effects of treatment. There was one significant effect of environment when rats were pretreated with saline as EC control rats displayed attenuated locomotor activity compared to SC control rats, $F(1,90)=4.91$, $p<.05$, (Figure 9A).

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 compared to SC rats, as paired EC and unpaired EC rats had attenuated locomotor activity compared to paired, $F(1,90)=4.65$, $p<.05$, and unpaired, $F(1,90)=5.28$, $p<.05$, SC rats (Figure 9B).

Comparing saline and MTEP 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, $F(1,90)=4.11$, $p<.05$, and IC unpaired, $F(1,90)=4.65$, $p<.05$, rats compared to saline pretreatment.

Z-score standardization.

As with the conditioned hyperactivity test, because there were baseline differences between EC, IC, and SC rats during sensitization training, locomotor activity during the sensitization test was standardized (Figures 10A and 10B). Results revealed a main effect for environmental condition, $F(2,90)=9.49$, $p<.001$, a main effect for amphetamine treatment, $F(2,90)=5.60$, $p<.01$, and a main effect for MTEP treatment, $F(1,90)=15.10$, $p<.001$.

Sensitization test timecourse.

To analyze the effects of MTEP throughout the entire session, MTEP and saline treatment groups were compared during each 10 minute bin throughout the 60 minute sensitization test. Results did not reveal changes in MTEP's effectiveness during the majority of the sensitization session, though as with the conditioned hyperactivity test, it appeared that there were fewer MTEP/saline differences during the last few bins of the hour session (Table 3). MTEP attenuated locomotor activity in the majority of EC (Figure 11A) and IC (Figure 11B) paired and unpaired rats, while fewer differences were observed in EC and IC control rats, as well as SC paired and unpaired rats (Figure 11C).

Although sensitization was not observed overall during the sensitization test, when broken down into 10 minute bins, sensitization was observed. EC paired saline rats displayed greater locomotor activity than EC control saline rats during bins 1, 3, 4, and 5, $Fs(1,450)>6.28$, $ps<.05$. IC paired saline rats demonstrated greater locomotor activity than IC control saline rats during bins 1, 3, 4, 5, and 6, $Fs(1,450)>6.68$, $ps<.05$. SC paired saline rats had greater locomotor activity compared to SC control saline rats during bin 1, $F(1,450)=12.08$, $p<.001$. Following MTEP pretreatment sensitization was not observed in EC rats, though it was observed in IC and SC rats. IC paired MTEP rats displayed greater locomotor activity than IC control MTEP rats during bins 3 and 4, $Fs(1,450)=4.60$, $ps<.05$. SC paired MTEP rats had greater locomotor activity compared to SC control MTEP rats during bins 1 through 5, $Fs(1,450)>5.66$, $ps<.05$.

Discussion

Overall, results of the current study showed that MTEP significantly attenuated conditioned hyperactivity and sensitization in IC but not EC and SC rats. These findings suggest

that glutamatergic changes occur in EC, IC, and SC rats during rearing, which alter the behavioral effects of MTEP on amphetamine conditioning and sensitization.

Acquisition

Results of acquisition revealed that EC rats had attenuated locomotor activity compared to IC and SC rats during acquisition. This is consistent with previous research that suggests EC rats are less sensitive to the effects of amphetamine following repeated administration compared to IC rats (Bardo et al., 1995; Bowling & Bardo, 1994). Data also revealed that SC paired rats had greater locomotor activity compared to IC paired rats. While the behavior of SC rats is typically hypothesized to be between that of EC and IC rats, the results of the current study are consistent with previous findings in our laboratory which show that SC rats have greater amphetamine-induced locomotor activity compared to IC rats (Gill & Cain, 2008). As SC rats have demonstrated greater amphetamine-induced locomotor activity in multiple studies, this discrepancy is most likely because the majority of older studies on differentially reared rats only compared EC and IC rats (Bardo et al., 1995; Bardo et al., 2001; Bowling & Bardo, 1994). Additionally, paired rats demonstrated greater locomotor activity compared to unpaired and control rats which is also consistent with previous research, as amphetamine is a stimulant that causes increased locomotor activity (Mazurski & Beninger, 1987; Tirelli & Terry, 1998).

Conditioned Hyperactivity Test

Conditioned hyperactivity and MTEP.

In the current study we hypothesized that administration of MTEP prior to conditioned hyperactivity would attenuate conditioned hyperactivity in EC paired rats compared to IC and SC paired rats. This hypothesis was partially supported as we observed an attenuation of

conditioned hyperactivity in EC compared to SC paired MTEP rats, and though conditioned hyperactivity was attenuated in EC compared to IC paired MTEP rats, it was not significant. Interestingly though, conditioned hyperactivity was not observed in EC saline rats, while it was observed EC, IC, and SC MTEP 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.

The changes that occur during rearing that alter Pavlovian conditioning in EC, IC, and SC rats are significant as there are several clinical implications. As context specific cues are often responsible for drug relapse (Everitt, Dickinson, & Robbins, 2001; Robinson & Berridge, 1993; Stewart, de Wit, & Eikelboom, 1984), discovering the mechanisms behind conditioned hyperactivity, as well as protective factors for conditioned hyperactivity will help develop methods to prevent drug relapse. Conditioned hyperactivity occurs when rats in a drug free state are exposed to contextual cues previously paired with a drug. This context-drug pairing leads to a context specific increase in locomotor activity (Ahmed et al., 1998; Barr et al., 1983; Gold et al., 1988; Pickens & Crowder, 1967).

Several studies 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. Bowling and Bardo (1994) showed that EC rats have greater amphetamine CPP than IC rats at .5 and 2.0 mg/kg doses, and SC rats at a .5 mg/kg dose. Schenk, Hunt, Malovechko, Robertson, Klukowski, and Amit (1986) also observed greater cocaine CPP in EC rats compared to IC rats, though differences in CPP were not observed following low doses of amphetamine. Interestingly, Gehrke, Cass, and Bardo (2006) observed

CPP in IC but not EC rats following a low dose of methamphetamine. Zakharova and colleagues (2009) as well as Solinas and colleagues (2008) also showed attenuated cocaine CPP in enriched rats or mice following low to moderate doses of cocaine. Research from our laboratory revealed that both EC and IC rats display conditioned hyperactivity following a 0.3 mg/kg dose, while only EC rats demonstrate conditioned hyperactivity following a 1.0 mg/kg dose of amphetamine (Neises et al., 2006). Thus, previous studies reveal greater conditioned hyperactivity in EC compared to IC rats at high doses of psychostimulants, but no differences or attenuated conditioned hyperactivity using low to moderate doses of psychostimulants. In the current study we observed conditioned hyperactivity following a 0.3 mg/kg dose of amphetamine in IC, but not EC rats. This suggests that environmental enrichment may have a protective effect against conditioned hyperactivity, and thus, relapse with Pavlovian conditioned cues when trained using low to moderate doses of psychostimulants. Additionally, this is the first study to demonstrate an enrichment-induced protective effect using a low dose, 0.3 mg/kg, of amphetamine. This is an important finding as it shows that enrichment may protect against drug relapse from the start, as most people begin abusing drugs at low doses. Additionally, these results suggest that rearing may protect against drug relapse in humans.

As conditioned hyperactivity was observed in EC MTEP, as well as IC and SC, MTEP and saline rats, while it was not observed in EC saline rats, it suggests that conditioned hyperactivity in EC rats may occur due to mGluR5 function. Kim, Vezina, and Kim (2008) work confirms the results of the current study, and particularly those of the standard housed rats. Results revealed that conditioned hyperactivity was not attenuated by group I mGluR antagonists, though conditioned hyperactivity was attenuated by group II mGluR antagonists. As mGluR5 is

a group I mGluR antagonist subtype, and rats were standard housed in Kim and colleagues' study, results confirm the findings of the current study. However, Kumaresan and colleagues (2009) as well as Bäckström & Hyytiä (2007) showed an attenuation of cue-induced reinstatement following MTEP and MPEP administration respectively, when rats were individually or socially housed. This suggests more similarities are present between IC and SC rats than differences. While no previous studies have investigated the effect of mGluR antagonists on enriched rats, the current study revealed differences in conditioned hyperactivity of enriched rats due to mGluR5 function. These results suggest that changes occur in enriched rats during rearing that contribute to these glutamatergic differences during conditioned hyperactivity. A few areas that may contribute to these glutamatergic differences include the NAcc, VTA, and hippocampus.

Neurobiological mechanisms.

Nucleus accumbens.

Research suggests that the NAcc plays a significant role in glutamatergic differences during Pavlovian conditioning in enriched, but not standard housed rats. Kim et al., (2008) demonstrated that standard housed rats do not have glutamatergic differences in the NAcc, as they did not observe any behavioral changes when they microinjected a group I mGluR antagonist, AIDA, into the NAcc prior to the conditioned hyperactivity test. Additionally, Bell, Duffy, and Kalivas (2000) did not observe differences in glutamate in the NAcc of standard housed rats, using microdialysis during the conditioned hyperactivity test. As in the current study, these studies suggest that mGluR5 function is not involved in conditioned hyperactivity of standard housed rats.

Though nobody has investigated the role of mGluR5 in psychostimulant conditioning of differentially reared rats, mGluR1 does not appear to influence conditioning in differentially reared rats. Wood and colleagues (2005) found that EC rats have reduced NMDA receptors compared to IC rats in the NAcc, while there are no differences in mGluR1 expression in the NAcc of EC and IC rats. MGluR1 and mGluR5 both contribute to drug abuse (Satow et al., 2008), and long-term potentiation (LTP) as mGluR1 is down regulated by mGluR5 during LTP (Bikbaev et al., 2008). In fact, administration of mGluR5 antagonists produce LTP deficits, as well as inhibition of mGluR1 (Bikbaev et al., 2008). During psychostimulant use, drug seeking may become more persistent as mGlu1 receptors dissociate from Homer scaffolding proteins in the VTA, causing greater cocaine-induced synaptic plasticity, and greater synaptic plasticity in the NAcc (Mameli et al., 2009; Ungless, Whistler, Malenka, & Bonci, 2001). Thus, while Bikbaev and colleagues' (2008) results may suggest a lack of mGluR5 expression in the NAcc, Mameli and colleagues (2009) as well as Ungless and colleagues' (2001) work suggests that mGluR5 may be expressed in the NAcc due to the synaptic plasticity that occurs during repeated psychostimulant use.

Ventral tegmental area.

While the role of the VTA in glutamate expression and conditioned hyperactivity has not been investigated in differentially reared rats, glutamate in the VTA does appear to be critical for Pavlovian conditioning. This was demonstrated as mice did not develop cocaine CPP when the VTA was injected with glutamate antagonists; however, cocaine CPP was present when glutamate antagonists were injected elsewhere (Harris & Aston-Jones, 2003). Additionally, glutamate may contribute to psychostimulant abuse in the VTA as mGluR1 detaches from

Homer scaffolding proteins in the VTA and induces greater cocaine-induced synaptic plasticity in the NAcc (Mameli et al., 2009).

Hippocampus.

Past research suggests the hippocampus contributes to plasticity during the differential rearing period, in addition to several learning and memory tasks (Renner & Rosenzweig, 1987). The hippocampus is altered by environmental enrichment, as rearing rats in an enriched environment has been shown to increase the number of granule cells in the dentate gyrus (Susser & Wallace, 1982), increase the thickness and density of glial cells (Kempermann, Kuhn, & Gage, 1997; Walsh, Budtz-Olsen, Penny, & Cummins, 1969), increase the dendritic branching and the size of the dendritic field in the dentate gyrus (Fiala, Joyce, & Greenough, 1978), increase synaptic density in the CA3 area of the hippocampus (Altschuler, 1979b), and enhance the synaptic strength of dentate gyrus and pyramidal cells in the CA1 area of the hippocampus (Foster & Dumas, 2001; Green & Greenough, 1986a, 1986b). These differences in enrichment-induced plasticity in turn alter glutamatergic levels in EC and IC rats as Segovia and colleagues (2006) measured glutamate levels in the CA3 region of the hippocampus of young and old, enriched and impoverished rats. While no differences in glutamate basal dialysate concentrations were observed in young enriched and impoverished rats, old enriched rats had greater concentrations of glutamate compared to old impoverished rats.

While Segovia and colleagues (Segovia et al., 2006) did not observe differences in glutamate in EC and IC young rats, it may be because they only examined glutamate in the CA3 region of the hippocampus. Researchers have shown that there are two separate pathways for different types of long-term potentiation (LTP) in the hippocampus, one involving the CA3

region and another involving the CA1 region (Lu et al., 1997; Nicoll & Malenka, 1995). LTP in the CA3 region of the hippocampus occurs primarily in pre-synaptic glutamatergic receptors, and not post-synaptic glutamatergic receptors, such as mGluR5 (Lu et al., 1997; Nicoll & Malenka, 1995). However, mGluR5 is the primary receptor involved in LTP in the CA1 area, while mGluR1a is absent (Baude et al., 1993; Conquet et al., 1994; Lu et al., 1997; Shigemoto et al., 1993). This was demonstrated as Lu and colleagues (1997) found mGluR5 to be essential for NMDA receptor LTP in the CA1 area of the hippocampus and the perforant pathway as mGluR5 deficient mice display attenuated LTP in these areas. Manahan-Vaughan and Braunewell (2005) found similar results as MPEP attenuated LTP in the CA1 region and dentate gyrus of rats. Thus, as the current study investigated the role of mGluR5, we hypothesize that there may be glutamatergic differences in the CA1 region of the hippocampus in young EC and IC rats. Support for our hypothesis includes differences in hippocampal LTP of differentially reared rats. Researchers have observed an attenuation of LTP in the dentate gyrus and CA1 area of the hippocampus of IC compared to SC rats (Lu et al., 2003; Roberts & Greene, 2003). However, Ashby, Habib, Dringenberg, Reynolds, and Beninger (2010) did not observe differences in LTP in the CA1 area of IC and SC rats. In Ashby and colleagues' study researchers electrically stimulated the CA3 region of the hippocampus of IC and SC rats, and measured differences in LTP in the CA1 area of the hippocampus. Results revealed no differences in LTP of IC and SC rats in the CA1 region. This discrepancy in results is most likely due to the area that LTP is being induced. Due to the different pathways in the CA1 and CA3 areas of the hippocampus, in addition to differences in the type of glutamate receptors in these areas, these results suggest that induction of LTP in the CA3 area does not produce LTP in the CA1 area.

As the hippocampus contributes to plasticity and LTP in differentially reared rats, it has also been shown to influence psychostimulant-induced Pavlovian conditioning. Tzshentke and Schmidt (1997) showed that the hippocampus and glutamate impact conditioning, as MK-801 blocked amphetamine CPP when it was administered prior to the training session. Kelley et al., (2007) replicated these results and suggested that the MK-801 is blocking the reconsolidation of memory. Finally, as the ventral hippocampus has been shown to process contextual cues associated with aversive stimuli (Hobin, Ji, & Maren, 2006; Rudy & Matus-Amat, 2005) and drug seeking behavior (Vorel, Liu, Hayes, Spector, & Gardner, 2001), it most likely contributes to amphetamine-induced conditioned hyperactivity. The ventral hippocampus contributes to psychostimulant conditioning as it has been implicated in the processing of contextual cues associated with cocaine self-administration (Rogers & See, 2007; Sun & Rebec, 2003). However, despite the data supporting the role of the hippocampus in drug seeking and contextual conditioning, Black, Green-Jordan, Eichenbaum, and Kantak (2004) did not observe differences in cue-induced reinstatement when the dorsal or ventral subiculum were inactivated. Nor were differences observed between cocaine and saline paired cues during cue-induced reinstatement following inactivation of the ventral hippocampus (Atkins, Mashhoon, & Kantak, 2008). Thus, while the hippocampus contributes to pscyhostimulant-induced conditioned hyperactivity, there are most likely other areas or pathways involved.

Summary of neurological mechanisms during conditioned hyperactivity.

While there is support for the role of glutamate in all three brain areas during conditioned hyperactivity of differentially reared rats, which area or areas are necessary and/or sufficient is unclear. Though there are no studies investigating the role of mGluR5 in the NAcc

of enriched rats, previous studies show there are not glutamatergic differences in the NAcc of standard housed rats. While there is little evidence of the role of mGluR5 in the VTA of differentially reared rats, due to the role of glutamate in the VTA during Pavlovian conditioning as well as cocaine-induced synaptic plasticity there is a good likelihood that mGluR5 is involved in conditioned hyperactivity in the VTA. Research also supports the involvement of mGluR5 in the hippocampus during conditioned hyperactivity of differentially reared rats. Researchers have revealed greater concentrations of glutamate in EC compared to IC rats, and MPEP is shown to attenuate LTP in the CA1 region of the hippocampus. Thus, while there is evidence of involvement in all three areas, it is unknown whether it is one or several areas that contribute to glutamatergic differences in differentially reared rats.

Differential rearing and conditioned hyperactivity.

In the current study we hypothesized that paired rats that were administered MTEP prior to conditioned hyperactivity would display attenuated conditioned hyperactivity compared to paired rats that did not receive MTEP. Results revealed that MTEP effectively attenuated conditioned hyperactivity in all IC treatment groups, however, it did not attenuate conditioned hyperactivity in EC treatment groups, or SC paired or unpaired treatment groups. As MTEP primarily attenuated conditioned hyperactivity in IC rats, this would suggest that the social interactions in SC rats may have a slight protective effect, but not to the extent of enriched rats (Bardo & Dwoskin, 2004; Renner & Rosenzweig, 1987). Previous research suggests that novel objects, handling, and social rearing are all needed to produce a protective effect (Bardo & Dwoskin, 2004; Renner & Rosenzweig, 1987), and this was observed in the current study as we saw an attenuation of conditioned hyperactivity in EC compared to IC and

SC saline rats. As the current results are not in accordance with original differential rearing research, and much of the differential rearing research since then has only used EC and IC rats, these results suggest that it may be beneficial to parse out the differences in different rearing conditions in the future when psychostimulants are involved.

The results of the current study also confirm that the observed effects are not just a result of baseline differences in EC rats, as paired EC MTEP rats displayed greater locomotor activity than paired EC saline rats, as well as EC unpaired and control MTEP rats. If observed effects were just due to baseline differences, conditioned hyperactivity would not be observed following the standardization of data. However, conditioned hyperactivity was still observed in EC MTEP rats. Additionally, the current results suggest the observed effects are not due to non-specific effects of MTEP, as conditioned hyperactivity was observed in EC, IC, and SC rats administered MTEP. If MTEP was abolishing locomotor activity, motor activity in all of the treatment groups would be attenuated, and conditioned hyperactivity would not be observed. Thus, results of conditioned hyperactivity reveal that rearing alters mGluR5 functioning, which in turn influences conditioned hyperactivity. While the specific areas of mGluR5 action are unknown, it is likely that several areas of the glutamatergic projection are necessary for the development of conditioned hyperactivity. As rearing induced changes to the glutamatergic pathway alter the presence of conditioned hyperactivity, with further research into the mechanisms that cause these glutamatergic changes, researchers may be able to develop methods to prevent drug relapse in humans.

Sensitization Training

While the conditioned hyperactivity test investigated the potential role of mGluR5 in drug relapse, the sensitization test investigated the role of mGluR5 in drug sensitivity. Results of sensitization training were similar to those of acquisition as EC rats had attenuated locomotor activity compared to IC and SC rats, and paired rats displayed greater locomotor activity compared to unpaired and control rats. One difference in acquisition and sensitization training was that IC and SC paired rats did not differ in locomotor activity during sensitization training as they did during acquisition. As mentioned earlier, researchers typically hypothesize that the behavior of SC rats will be more similar to IC rats due to similar rearing conditions. However, as observed in acquisition as well as previous studies this is not always the case.

Sensitization Test

Amphetamine sensitization.

We hypothesized that MTEP administration prior to the sensitization test would attenuate sensitization in paired rats and have no effect on unpaired and control rats. Interestingly, during the sensitization test, while sensitization was observed for the overall session, simple effects did not reveal any group differences. However, when data were broken down into 10 minute bins throughout the sensitization test, differences were observed during the first 10 minute bin in all groups except the IC MTEP rats. Unfortunately, the treatment effect was attenuated as the session progressed, thus, partially accounting for why there were no significant simple effects. The reason for the decrease in locomotor activity after the first 10 minute bin could be due to the low dose of amphetamine used, as past studies have used much higher doses of amphetamine (McGeehan & Olive, 2003; Parelkar & Wang, 2004). In the

current study we used a low enough dose to observe differences in sensitization between EC, IC, and SC rats, as EC, IC, and SC differences are attenuated following a moderate to high dose of amphetamine. However, the dose may have been too low as amphetamine-induced locomotor activity was attenuated after the first 10 minute bin during the sensitization test. The lack of effect throughout the entire hour session most likely is not due to the metabolism of MTEP during the session as research has shown that a 3 mg/kg, i.p. dose of MTEP attains 100% receptor occupancy within minutes and decreases to near zero occupancy within 4 hours. Additionally, MTEP maintains greater than 75% receptor occupancy in the rat brain for 2 hours following a 3 mg/kg, i.p. dose. When dose-response studies were conducted, results revealed an ED_{50} of 60 minutes in rats (Andersonet al., 2003). Further, MTEP has a 16% bioavailability and a half maximal inhibitory concentration (IC_{50}) of 5 nM (Cosford et al., 2003), and a half-life ($t_{½}$) of 8 hours (Green, Yang, Cramer, & King, 2006). Thus, the lack of treatment effect is most likely due to the doses of amphetamine and MTEP, not the effectiveness of either.

MTEP pretreatment.

Despite previous studies that have shown sensitization of differentially reared rats using a 0.3 mg/kg dose of amphetamine (Bardo et al., 1995; Neises et al., 2006), the current study did not observe sensitization. This lack of sensitization following MTEP pretreatment may be due to changes in mGluR5 following repeated amphetamine administrations. This hypothesis is based on work by Hao, Martin-Fardon, and Weiss (2010) who demonstrated that mGluR5 expression decreased as rats became cocaine dependent. mGluR5 was measured in the NAcc, PFC, and hippocampus following training on a short or long access cocaine self-administration task. When administered MTEP, the long access group displayed decreased mGluR5 expression

in the NAcc, and trended toward decreased expression in the hippocampus and PFC compared to the short access group. Thus, if mGluR5 levels decrease as rats become cocaine dependent, in the current study one would expect a decrease in the effect of MTEP on sensitization compared to during conditioned hyperactivity if rats became dependent upon amphetamine.

In the current study we hypothesized that MTEP would attenuate locomotor behavior significantly more in EC compared to IC and SC paired rats. Interestingly, the opposite was observed as simple effects revealed that MTEP pre-treatment attenuated locomotor activity in IC paired and unpaired rats compared to saline pre-treatment. When broken into 10 minute bins, results revealed that MTEP was most effective at attenuating amphetmine-induced sensitization early in the session. For instance MTEP attenuated locomotor activity in all groups except EC control and EC paired rats during the first two 10 minute bins. Interestingly, MTEP attenuated locomotor activity in IC paired and unpaired rats in each bin. As with conditioned hyperactivity, these results suggest that changes occur in the glutamatergic pathway during rearing that alter the way amphetamine is processed in EC compared to IC rats. Additionally, results of environmental rearing produced attenuated locomotor activity in EC paired and unpaired rats compared to SC paired and unpaired rats. As these results were not observed in saline pretreated rats, nor were they altered following z-score transformation, they suggest that differences arise during differential rearing that alter the glutamatergic pathways in EC, IC, and SC rats, thus, altering the way amphetamine acts on the brain.

The changes in locomotor activity due to glutamate antagonists are congruent with previous research, which showed altered levels of glutamate in the brain following psychostimulant administration. Three specific areas are hypothesized to contribute to these

glutamatergic changes including the PFC, VTA, and NAcc. As previously mentioned, the mesolimbic DA system is responsible for the rewarding effects of psychostimulants, and it induces glutamate in a feedback loop that projects from the PFC to the VTA, and NAcc (Ghitza et al., 2004; Vanderschuren & Kalivas, 2000).

Prefrontal cortex.

Glutamatergic differences may begin in the PFC as mGluR5 protein expression increases in the PFC when rats are exposed to an acute dose of amphetamine (Shaffer, Guo, Fibuch, Mao, & Wang, 2010). mGluR5 expression in the NAcc also appears to be influenced by differential rearing, as EC rats have significantly greater levels of mGluR5 dimers in the PFC compared to IC rats, while there are no differences in mGluR5 monomers in EC and IC rats, both when no drugs are present (Melendez et al., 2004) and following an acute amphetamine injection (Shaffer et al., 2010). While glutamate expression in the PFC appears to be involved in amphetamine-induced behavior following acute administration, it does not appear to be entirely responsible for sensitization as Kozell and Meshul (2001) did not observe differences in glutamate immunolabeling in rats that received an acute cocaine injection compared to those sensitized to cocaine. This was also demonstrated by Williams and Steketee (2004) as they did not observe changes in glutamate expression in the PFC during the sensitization test if the rats had between a 7 and 30 day sensitization period. Thus, while mGluR5 levels may differ in the PFC of EC, IC, and SC rats, it is most likely not completely responsible for differences in sensitization in differentially reared rats.

Ventral tegmental area.

Glutamatergic differences have also been observed in the projections from the PFC to the VTA as the VTA has been shown to be involved in plasticity and psychostimulant sensitization. Bird and colleagues (2010) demonstrated the role of the mGluR5 and the VTA in cocaine-induced plasticity as they used a AMPA/NMDA/EPSC ratio to show that mGluR5 is required in the VTA for cocaine induced plasticity following an acute cocaine injection. Using this ratio, Bird and colleagues (2010) also claim that the VTA is not essential for behavioral sensitization; however, other work shows differences in glutamate expression in the VTA following sensitization. Kozell and Meshul (2001) demonstrated that glutamate immunolabeling was increased in the VTA of rats sensitized to cocaine, compared to those that received an acute cocaine injection. Additionally, several studies have shown that intra-VTA administration of amphetamine produces sensitization (Cador, Bjijou, & Stinus, 1995; Hooks, Jones, Liem, & Justice, 1992; Kalivas & Weber, 1988; Perugini & Vezina, 1994; Vezina, 1996; Vezina & Stewart, 1990), and sensitization is blocked by intra-VTA administration of a glutamate antagonist (Cador, Bjijou, Cailhol, & Stinus, 1997; Kalivas & Alesdatter, 1993; Kim & Vezina, 1998). Wolf and Xue (1999) also showed that glutamate is involved in sensitization in the VTA, as they demonstrated increases in glutamate following each amphetamine injection (Xue et al., 1996). These glutamatergic increases may create a cascade of neural changes that allow for the induction of sensitization (Wolf & Xue, 1999). The differing results on the contribution of VTA glutamate are most likely due to the specificity of quantifying mGluR5 in Bird and colleagues' study (2010), while the other studies quantified general glutamate levels. As there is only one study investigating mGluR5 expression during sensitization, and no studies investigating the

role of mGluR5 in the VTA of differentially reared rats, future studies are needed to determine the role of mGluR5 in sensitization.

Nucleus accumbens.

As the PFC projects to the NAcc, it is not surprising that there are differences in glutamate in the NAcc of both standard housed and differentially reared rats. Glutamatergic changes following psychostimulant administration have been demonstrated in standard housed rats as several studies have observed increased levels of glutamate in the NAcc (Bell et al., 2000) and striatum (Del Arco et al., 1999; Reid & Berger, 1996; Xue et al., 1996). In contrast, Shaffer and colleagues observed an attenuation of mGluR5 monomer and dimer proteins in the striatum following an acute amphetamine administration (Shaffer et al., 2010). Following acute amphetamine administration, glutamatergic differences in the NAcc of differentially reared rats can be observed as Rahmen and Bardo (2008) showed greater levels of glutamate in the NAcc of EC rats compared to IC rats following treatment. These differences were not apparent following saline administration, as Melendez and colleagues (2004) did not observe differences in mGluR5 monomers and dimers in the striatum.

Summary of neurological mechanisms during sensitization.

As psychostimulant administration induces a glutamatergic feedback loop that projects from the PFC to the VTA and NAcc, it is likely that all three areas impact mGluR5 expression during sensitization of differentially reared rats. In the prefrontal cortex research reveals that there are glutamate differences in differentially reared rats following an acute amphetamine administration, but no studies have investigated the role of mGluR5 in sensitization of differentially reared rats. While no studies have investigated the role of glutamate in the VTA

of differentially reared rats, mGluR5 appears to be essential for plasticity following acute amphetamine exposure. Additionally, the majority of research supports the role of mGluR5 in sensitization of standard housed rats. The NAcc appears to contribute to differences in differentially reared rats as EC rats have shown greater levels of glutamate compared to IC rats. However, as previously mentioned, there are no studies investigating the role of mGluR5 in sensitization of differentially reared rats.

Future Directions

In the current study conditioned hyperactivity was observed in IC and SC saline, but not EC saline rats. As conditioned hyperactivity was only observed in EC MTEP rats, and not EC saline rats, results suggest that conditioned hyperactivity in EC rats may occur due to mGluR5 function. Thus, in the future it may be beneficial to use a higher dose of amphetamine that produces conditioned hyperactivity in all EC rats. By administering MTEP to EC rats that demonstrate conditioned hyperactivity, researchers could determine whether MTEP differentially effects conditioned hyperactivity in EC, IC, and SC rats.

Sensitization results revealed an overall amphetamine treatment effect, however, simple effects did not reveal sensitization in MTEP rats, nor was there an attenuation of locomotor activity in rats administered MTEP prior to the sensitization test. According to Hao and colleagues (Hao et al., 2010), this lack of sensitization occurs when rats become dependent upon cocaine, as mGluR5 levels decrease when rats become cocaine-dependent. Thus, in the future it may be beneficial to investigate sensitization in rats without extended amphetamine exposure. By eliminating the conditioned hyperactivity test, rats would only be exposed to amphetamine for 5 sessions prior to the sensitization test.

Additionally, as Hao and colleagues (Hao et al., 2010) showed a decrease in mGluR5 levels as rats became cocaine dependent, it would be interesting to investigate both the levels of mGluR5 and the behavioral effects during conditioned hyperactivity and sensitization if MTEP was administered during acquisition. By inhibiting mGluR5 expression during acquisition it may be possible to inhibit these mGluR5 changes, thus preventing conditioned hyperactivity and sensitization.

In the current study, only used Sprague-Dawley rats were used. Sprague-Dawley rats were chosen as an outbred strain is ideal for preliminary investigation, and it is the standard strain used in differential rearing research. Additionally, as we were investigating whether changes occur in glutamatergic pathways during rearing, it was important to use a standard strain in which the glutamatergic pathways could be altered. As the current study revealed that mGluR5 pathways are altered during rearing, thus, influencing contextual conditioning and sensitization, future studies will allow for the use of genetically altered mice such as mGluR5 knockout mice, or siRNA technology to investigate the effects of differential rearing on those mGluR5 pathways more specifically.

Conclusion

The current study suggests that mGluR5 is involved in both conditioned hyperactivity and sensitization in differentially reared rats. While results imply that mGluR5 impacts learning and memory during Pavlovian conditioning to a greater extent than plasticity during sensitization, the effects of MTEP on amphetamine-induced sensitization could be due to the development of amphetamine dependence following repeated exposure to amphetamine (Hao et al., 2010). Additionally, the results of the current study suggest that differential rearing

alters the glutamatergic pathways, and past research suggests that there are different mechanisms in the brain responsible for Pavlovian conditioning and sensitization (Bardo & Bevins, 2000), thus, why we observed different results during conditioned hyperactivity and sensitization. The current study suggests that differential rearing produces a protective effect during the development of plasticity that alters how mGluR5 acts in the brain, and thus, enhances learning and memory. However, further studies are still needed to pinpoint the exact brain areas and mechanisms involved. This has important clinical implications as it suggests that changes in mGluR5 pathways during enrichment rearing have a protective effect. With further research we may be able to determine what exact mGluR5 mechanisms are being altered and determine a way to prevent drug relapse and addiction.

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Figure Captions

Figure 1. Total locomotor distance (cm) traveled during acquisition in EC, IC, and SC, paired (1A) as well as unpaired and control (1B) 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. An asterisk (*) represents a significant difference between EC and IC/SC rats. A carrot (^) represents a significant difference between IC and SC paired or unpaired rats. A number sign (#) represents a significant difference between IC and SC control rats, $p<.05$.

Figure 2. Total locomotor distance (cm) traveled during acquisition in all environmental and treatment groups. All paired rats had significantly greater locomotor activity than unpaired and control rats. An asterisk (*) represents a significant difference between paired and unpaired/control EC, IC, and SC rats, $p<.01$.

Figure 3. Total locomotor distance during the final acquisition session for rats that would be assigned to MTEP and saline groups. This analysis was performed to confirm that there were no baseline differences.

Figure 4. Total locomotor distance (cm) traveled during the conditioned hyperactivity test for saline (4A) and MTEP (4B) rats. An asterisk (*) represents a significant difference between EC and IC/SC rats. A number sign (#) represents a significant difference between IC and SC rats. A carrot (^) represents a significant difference between paired and unpaired/control rats. A diamond (♦) represents a significant difference between MTEP and saline rats, $p<.05$.

Figure 5. Standardized z-scores of total locomotor distance (cm) traveled during the conditioned hyperactivity test for saline (5A) and MTEP (5B) rats. Refer to Figure 4 for

significant differences as significance values for the standardized locomotor activity are the same as for the non-standardized locomotor activity.

Figure 6. Total locomotor distance (cm) traveled during each 10 minute bin of the conditioned hyperactivity test for EC (6A), IC (6B), and SC (6C) rats. Refer to Table 2 for significant differences.

Figure 7. Total locomtotor distance (cm) traveled during sensitization training for paired (7A) and unpaired/control (7B) 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. An asterisk (*) represents a significant difference between EC and IC/SC rats, $p<.001$. A carrot (^) represents a significant difference between IC and SC paired or unpaired rats, $p<.05$. A number sign (#) represents a significant difference between IC and SC control rats, $p<.05$.

Figure 8. Total locomotor distance (cm) traveled during sensitization training. All paired rats had significantly greater locomotor activity than unpaired and control rats. An asterisk (*) represents a significant difference between paired and unpaired/control EC, IC, and SC rats, $p<.001$. A carrot (^) represents a significant difference between unpaired and control rats, $p<.05$.

Figure 9. Total locomotor distance (cm) traveled during the sensitization test for saline (9A) and MTEP (9B) rats. An asterisk (*) represents a significant difference between EC and IC/SC rats. A diamond (♦) represents a significant difference between MTEP and saline rats, $p<.05$.

Figure 10. Standardized z-scores of total locomotor distance (cm) traveled during the conditioned hyperactivity test for MTEP (10A) and Saline (10B) rats. Refer to Figure 9 for

significant differences as significance values for the standardized locomotor activity are the same as for the non-standardized locomotor activity.

Figure 11. Total locomotor distance (cm) traveled during each 10 minute bin of the sensitization test for EC (10A), IC (10B), and SC (10C) rats. Refer to Table 3 for significant differences.

Figures 1A & 1B

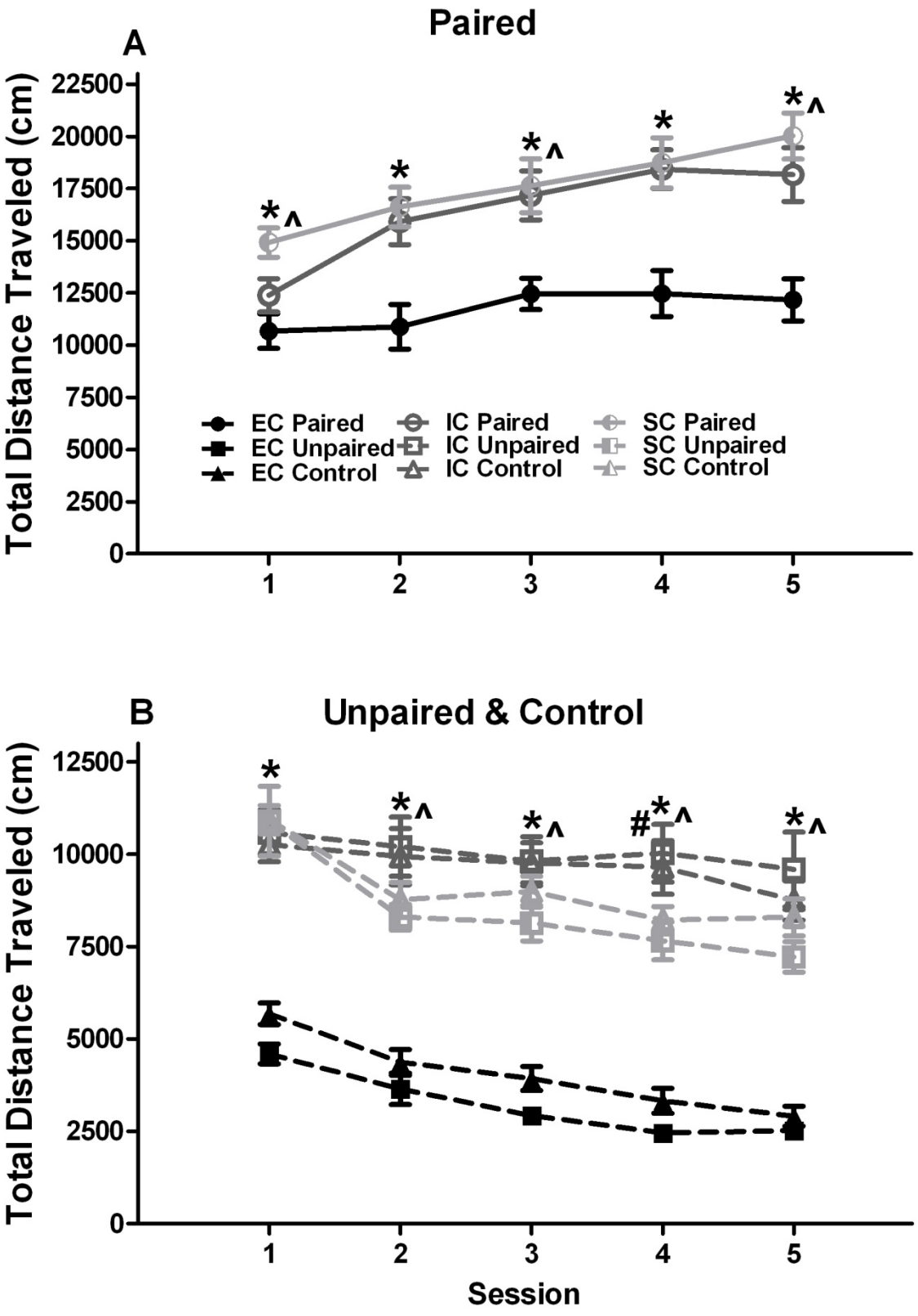


Figure 2

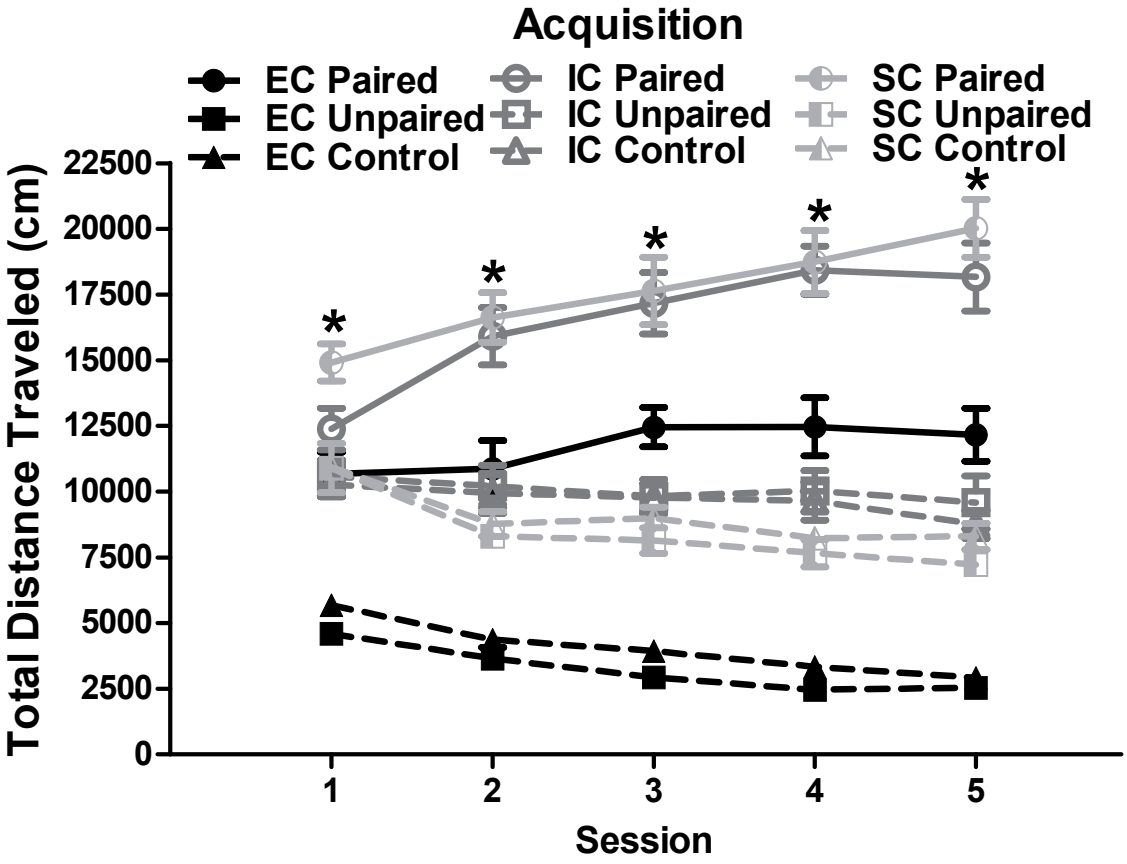
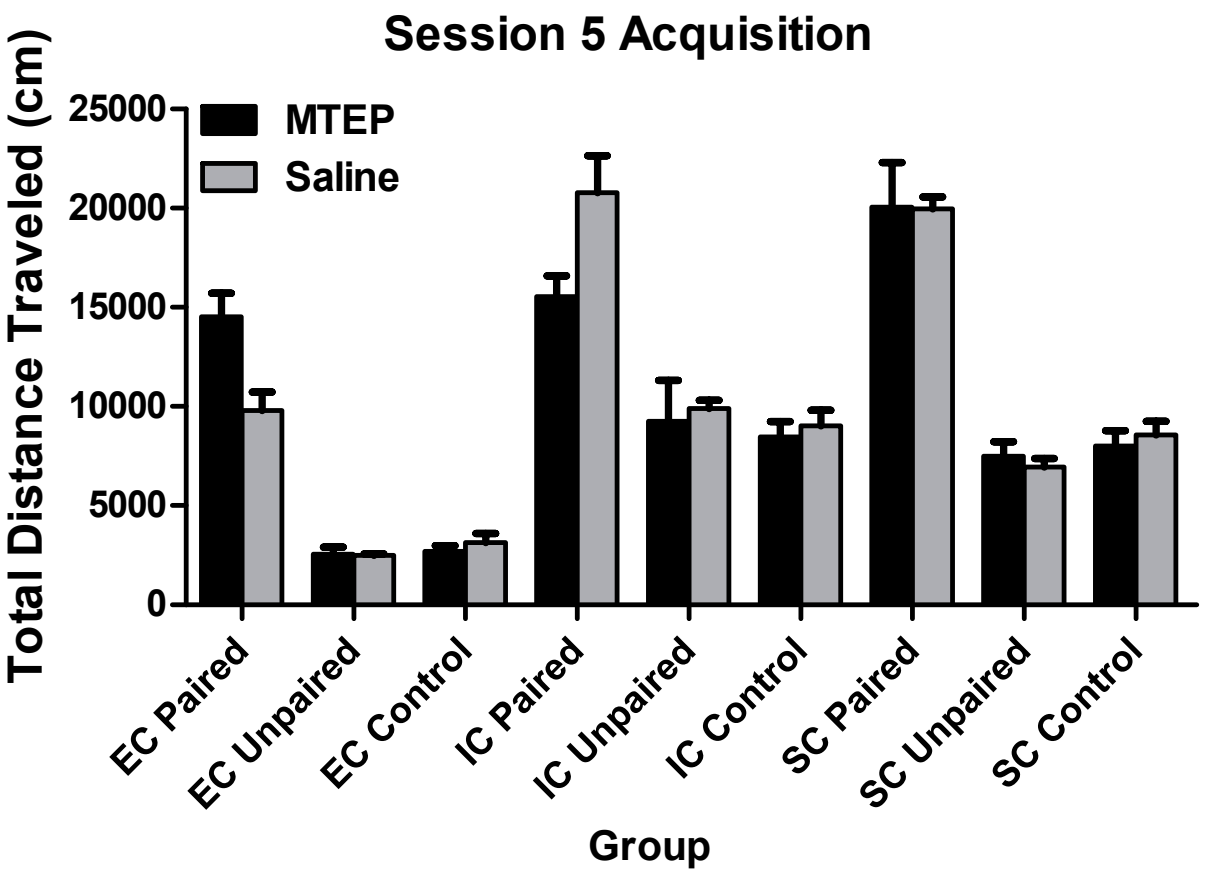
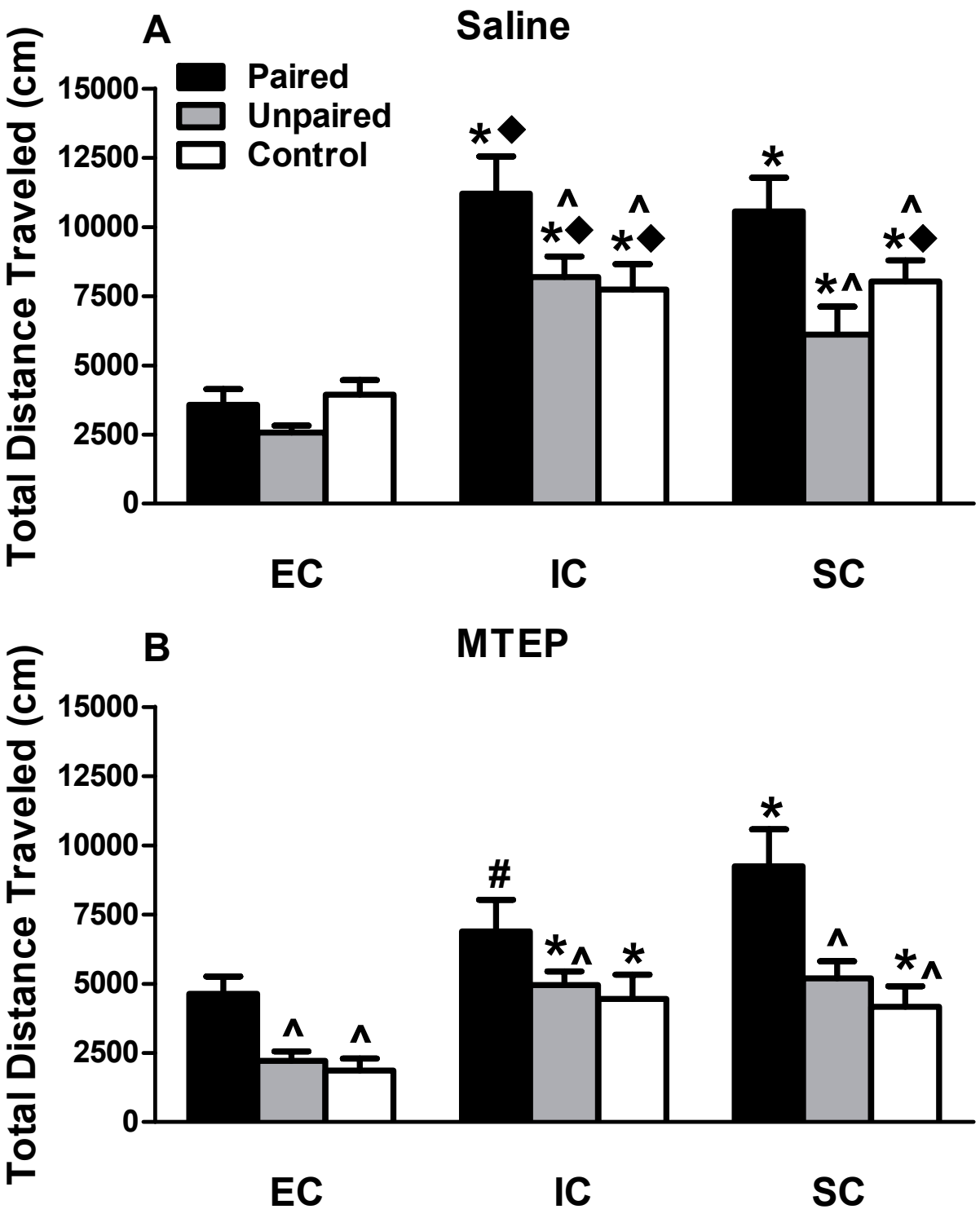

Acquisition
EC Paired
EC Unpaired
EC Control
IC Paired
IC Unpaired
IC Control
SC Paired
SC Unpaired
SC Control
Total Distance Traveled (cm)
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
1
2
3
4
5
Session
*
*
*
*
*

Figure 3


Session 5 Acquisition
Total Distance Traveled (cm)
25000
20000
15000
10000
5000
0
MTEP
Saline
EC Paired
EC Unpaired
EC Control
IC Paired
IC Unpaired
IC Control
SC Paired
SC Unpaired
SC Control
Group

Figures 4A & 4B



Figures 5A & 5B

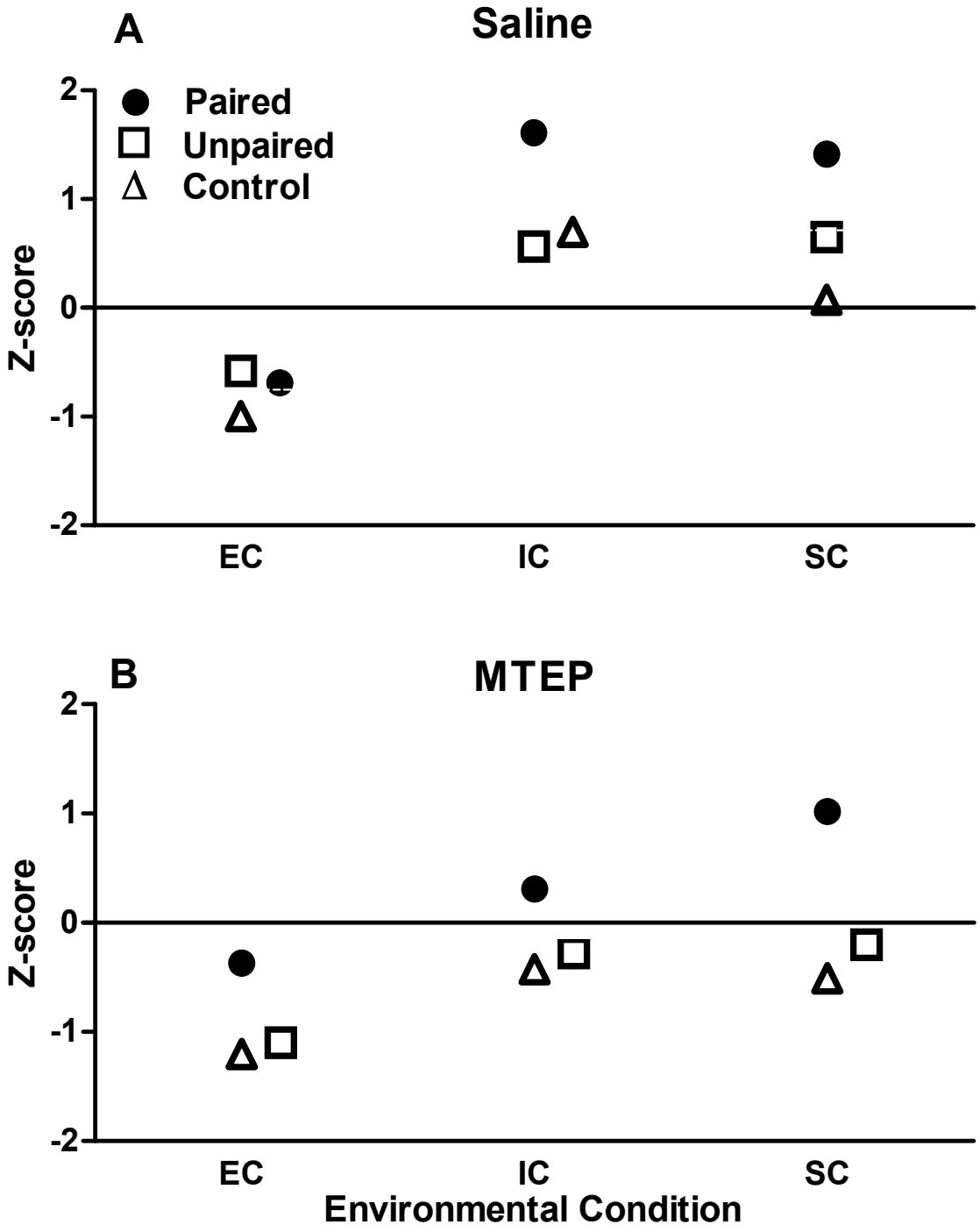
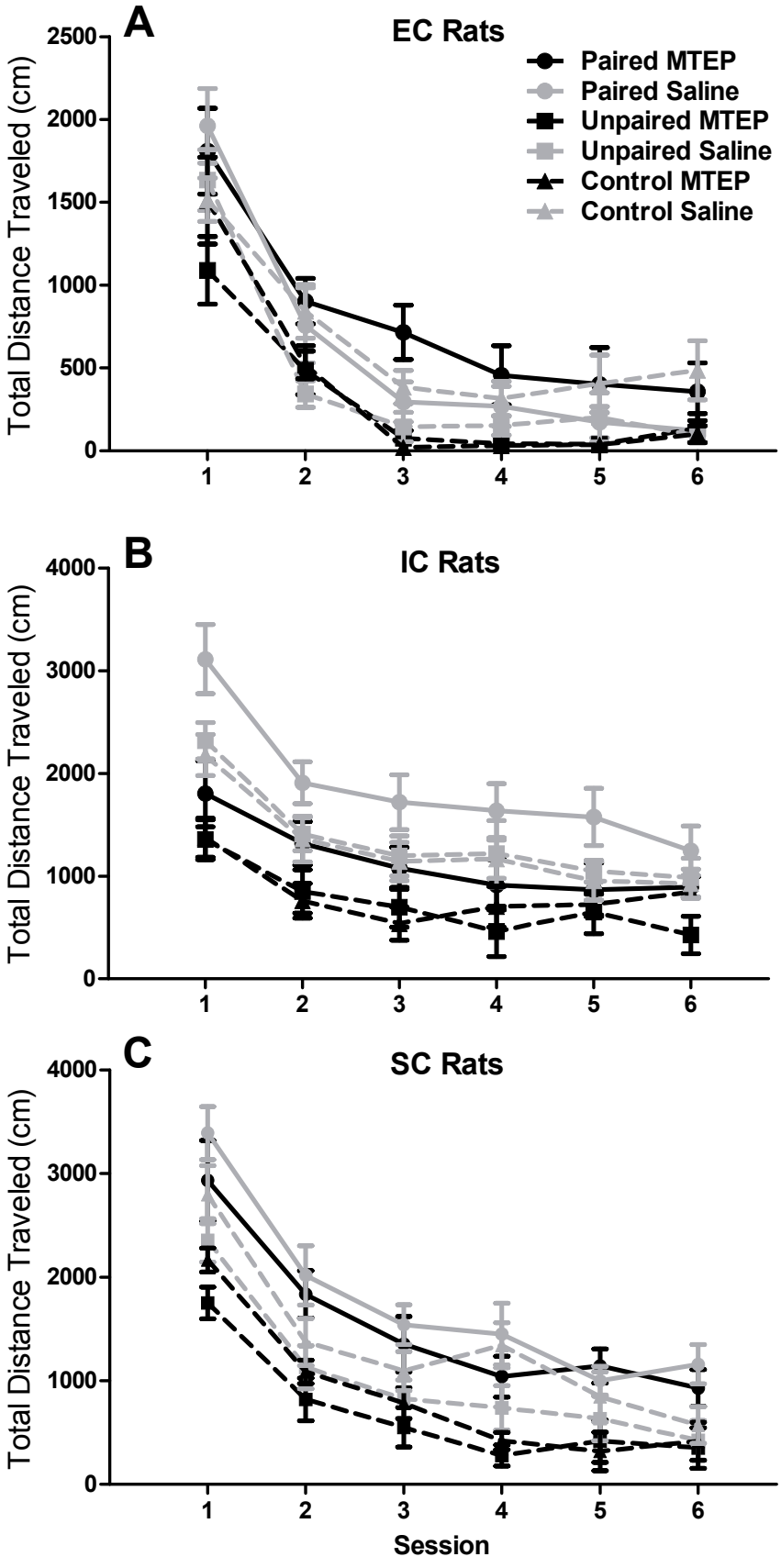


Figure 6


A
EC Rats
Paired MTEP
Paired Saline
Unpaired MTEP
Unpaired Saline
Control MTEP
Control Saline
Total Distance Traveled (cm)
2500
2000
1500
1000
500
0
1
2
3
4
5
6
B
IC Rats
Total Distance Traveled (cm)
4000
3000
2000
1000
0
1
2
3
4
5
6
C
SC Rats
Total Distance Traveled (cm)
4000
3000
2000
1000
0
1
2
3
4
5
6
Session

Figures 7A & 7B

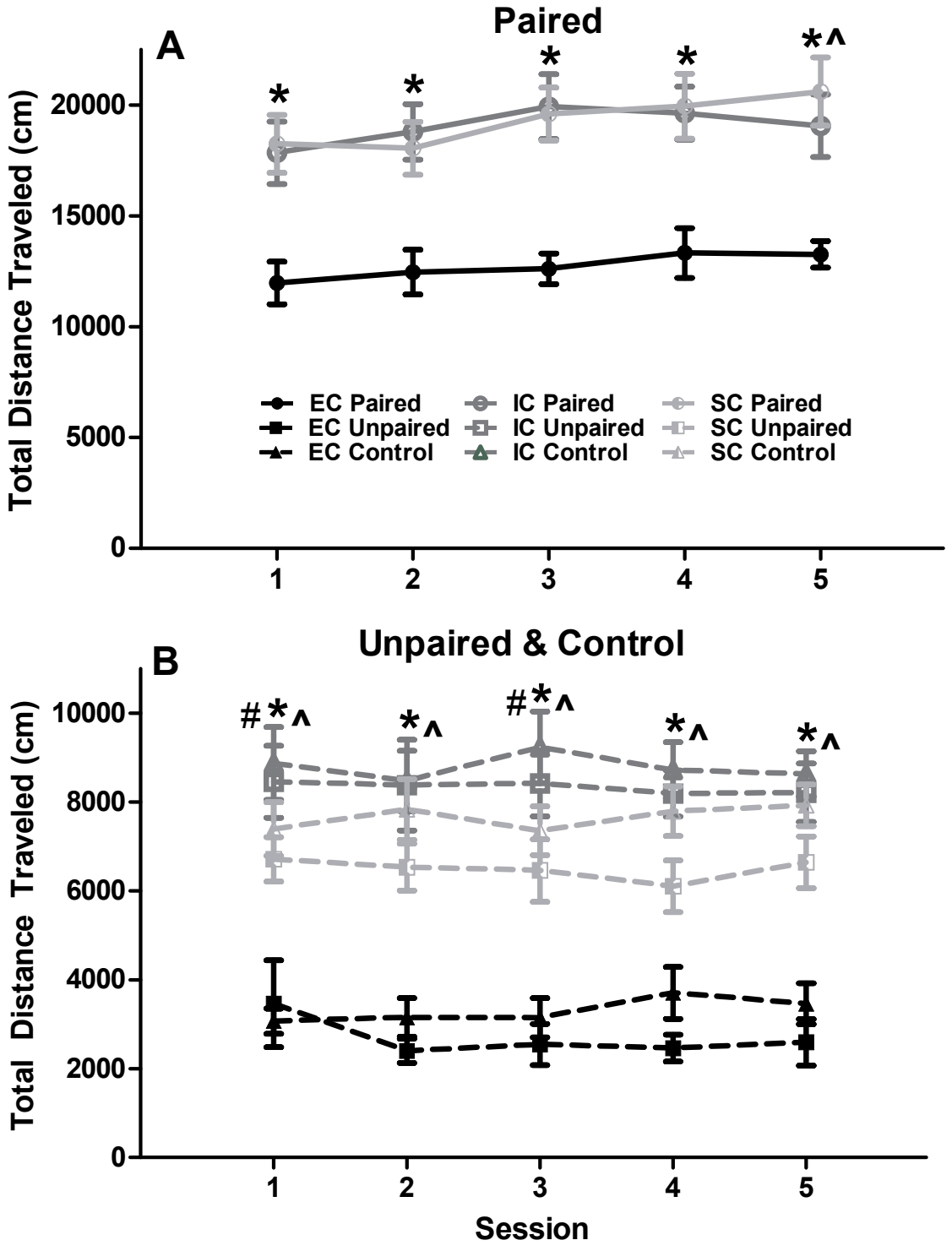
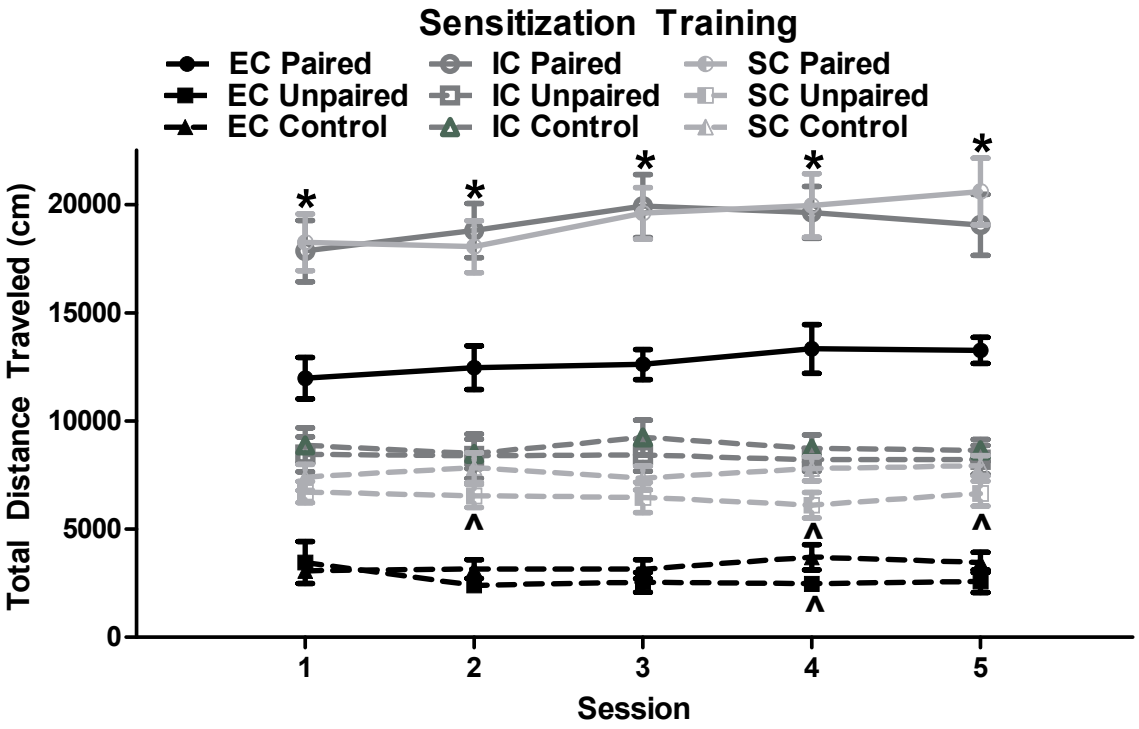
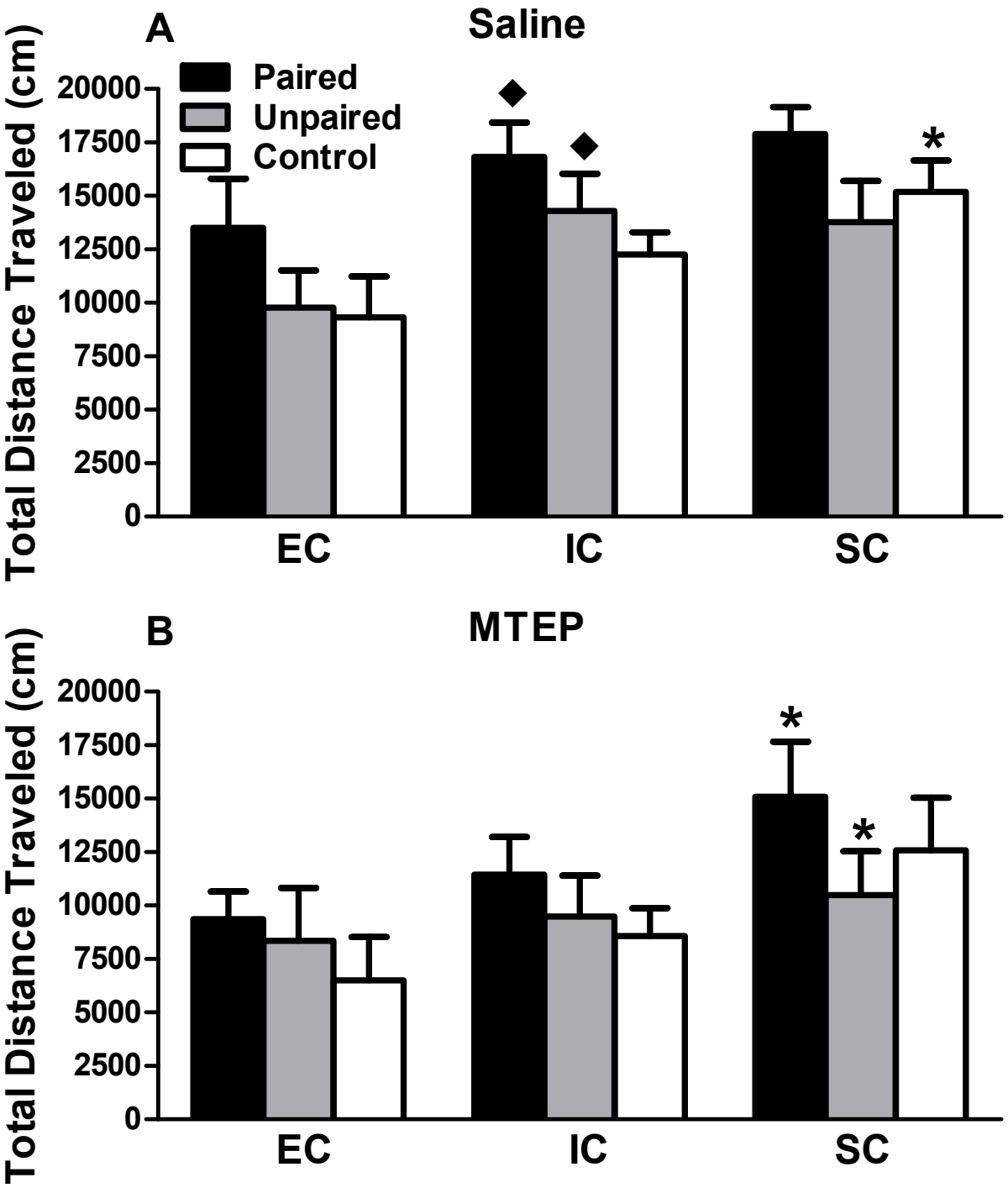


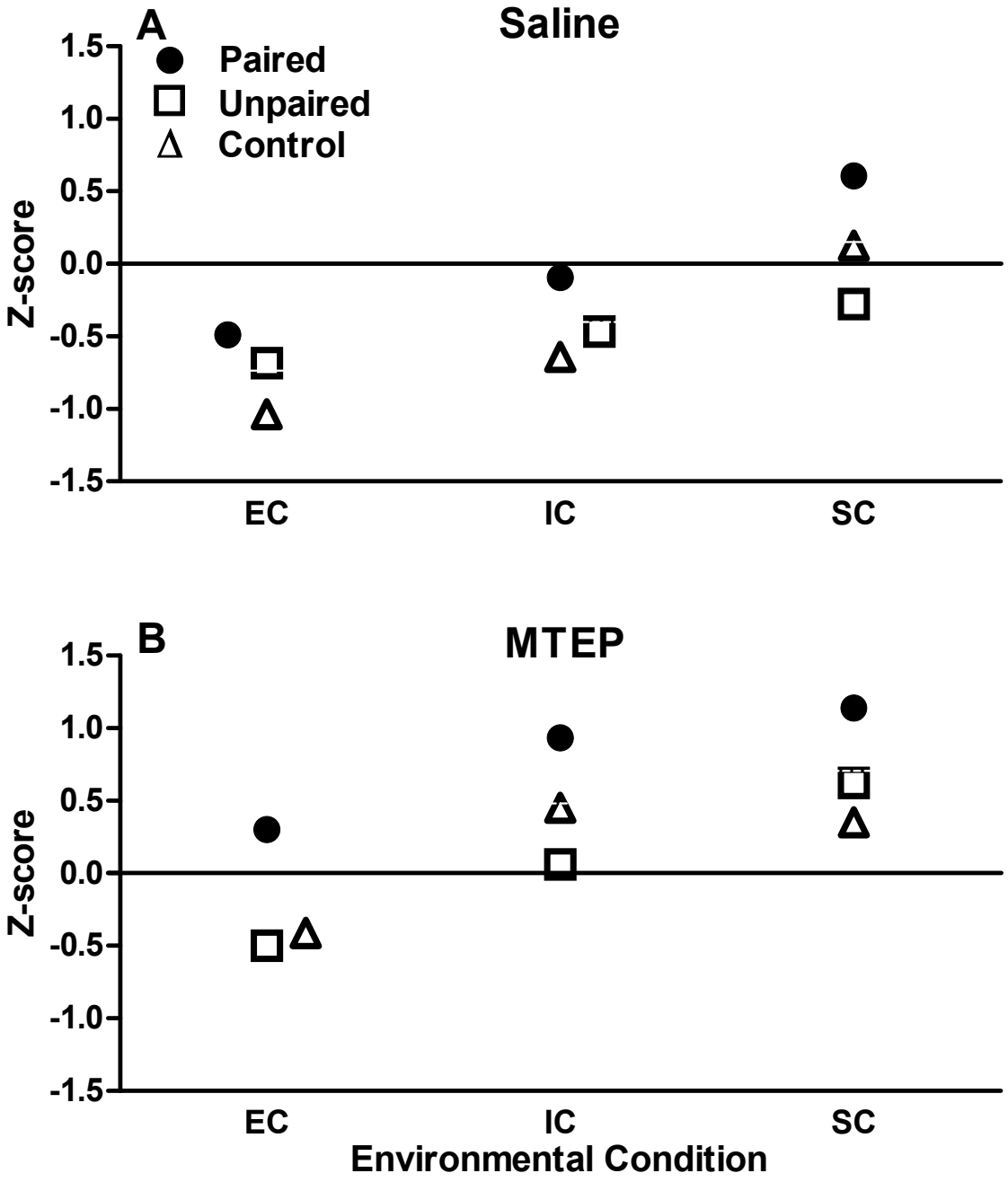
Figure 8


Sensitization Training
EC Paired
EC Unpaired
EC Control
IC Paired
IC Unpaired
IC Control
SC Paired
SC Unpaired
SC Control
Total Distance Traveled (cm)
20000
15000
10000
5000
0
1
2
3
4
5
Session
*
^

Figures 9A & 9B



Figures 10A & 10B



Figures 11A, 11B, & 11C

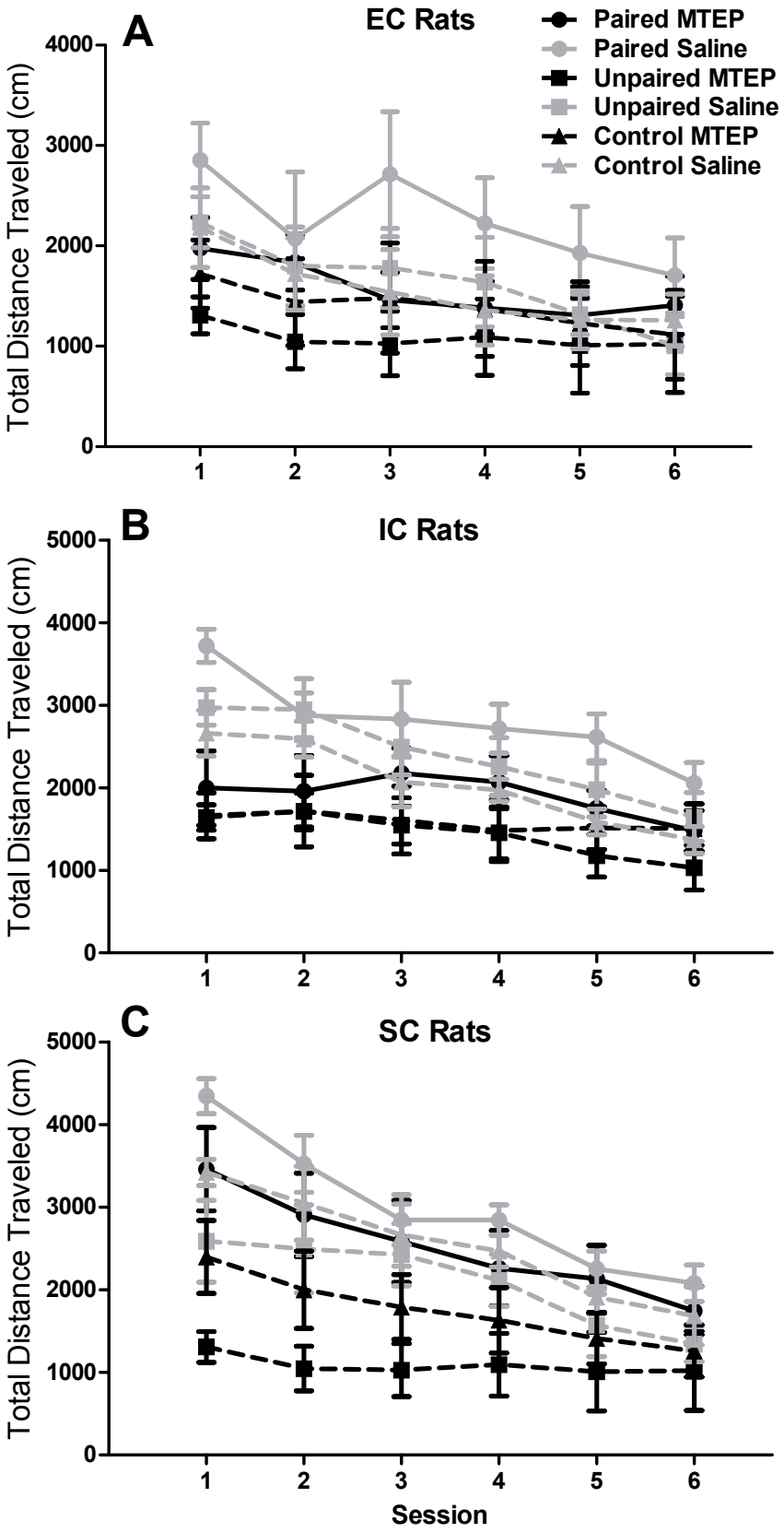


Table 1
Training schedule for EC, IC, and SC rats

	Conditioned Hyperactivity and Sensitization	
	MTEP	Saline
EC Paired	*n*=6	*n*=6
EC Unpaired	*n*=6	*n*=6
EC Control	*n*=6	*n*=6
IC Paired	*n*=6	*n*=6
IC Unpaired	*n*=6	*n*=6
IC Control	*n*=6	*n*=6
SC Paired	*n*=6	*n*=6
SC Unpaired	*n*=6	*n*=6
SC Control	*n*=6	*n*=6

Table 2
Multiple comparisons of timecourse during the conditioned hyperactivity test, data is presented in 10-minute bins.

			Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6
		df				F		
	Paired EC	1	0.58	0.53	4.42*	0.87	1.31	1.36
	Unpaired EC	1	7.41**	0.52	0.12	0.30	0.66	0.05
	Control EC	1	0.00	2.61	3.33	2.03	3.42	3.70
MTEP	Paired IC	1	42.89***	8.70**	10.36**	13.05***	12.57***	3.12
vs.	Unpaired IC	1	23.20***	7.79**	6.20*	14.34***	4.02**	7.79**
Saline	Control IC	1	16.16	9.08**	9.20**	5.34*	1.33	0.16
	Paired SC	1	5.25*	0.84	0.87	4.22*	0.50	1.32
	Unpaired SC	1	9.12**	2.39	1.88	5.29*	1.19	0.14
	Control SC	1	10.00**	2.07	2.42	21.26***	6.91**	0.61
	Error	450						

* $p<.05$, ** $p<.01$, ***$p<.001$

Table 3
Multiple comparisons of timecourse during the sensitization test, data is presented in 10-minute bins.

			Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6
		df	F					
	Paired EC	1	10.97***	0.78	22.26***	10.05**	5.45*	1.26
	Unpaired EC	1	12.16***	8.00**	8.04**	4.25*	1.37	0.00
	Control EC	1	3.00	1.14	0.05	0.00	0.02	0.30
MTEP	Paired IC	1	42.04***	11.94***	6.05*	6.00*	10.51**	4.68*
vs.	Unpaired IC	1	25.14***	21.50***	12.66***	9.08**	9.01**	5.35*
Saline	Control IC	1	14.16***	10.81**	3.03	3.39	0.08	0.27
	Paired SC	1	11.11***	5.41*	0.96	4.80*	0.20	1.58
	Unpaired SC	1	6.37*	4.48*	1.34	0.12	0.51	1.02
	Control SC	1	14.91***	15.58***	10.78**	9.98**	3.57	2.55
	Error	450						

* $p<.05$, ** $p<.01$, ***$p<.001$