EFFECTS OF DIFFERENTIAL REARING ON AMPHETAMINE-INDUCED C-FOS EXPRESSION IN RATS

by

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Abstract

Previous research has shown that both the environment and psychostimulant use influence dopamine levels via the mesolimbic dopamine pathway. *C-fos* expression has also been observed following exposure to novel environments and psychostimulants. The present study looked to determine the effects of acute amphetamine exposure on locomotor activity and *c-fos* expression in the basolateral and central nucleus of the amygdala, for rats raised in either an enriched condition (EC), impoverished condition (IC), or social condition (SC). Rats were reared in either the EC, IC, or SC for 30 days, after which they received an acute amphetamine injection (1.0 mg/kg) and locomotor activity was measured. Following the locomotor test rats were perfused and immunohistochemistry was used to measure *c-fos* levels in the basolateral and central nucleus of the amygdala. Results showed that EC amphetamine rats had significantly greater locomotor activity compared to EC saline rats. There were no significant group or treatment differences in *c-fos* expression in the ACe. In the BLA SC amphetamine rats had significantly greater *c-fos* expression than EC amphetamine rats. Overall, the current study revealed that environmental enrichment and amphetamine do significantly alter locomotor activity and *c-fos* expression in the BLA

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Introduction

The scientific community has recognized that both genetics and the environment are primary factors influencing human and animal traits and behaviors. Yet, many facets of human behavior continue to puzzle scientists as to the inherent and/or environmental origins. One of these multi-faceted behaviors that researchers continue to investigate is drug addiction. Researchers and doctors alike still scramble for explanations of drug abuse. Why is it that some people become addicted to drugs after just one use, while other people can use the same drug several times and never become addicted? Several researchers are working on tackling this debate by investigating how particular environmental factors alter behavior. There is much evidence that suggests the environment influences behavior neurologically and chemically. Specifically, past research suggests that neurological and chemical changes in the brain are influenced by the environment in which one is raised. Given the ethical boundaries of both drug and neurological research in humans, an animal model known as the enrichment paradigm has been created to model human behavior. Using the enrichment paradigm researchers can begin to investigate the effects of differential rearing on future drug use. The enrichment paradigm will assist researchers in determining what brain mechanisms are altered due to environmental upbringing, and how this influences drug abuse.

Enrichment

The original enrichment paradigm experiments consisted of two basic contexts, an impoverished condition and an enriched condition (Renner & Rosenzweig, 1987). Contexts within the enrichment paradigm vary as some researchers use environmentally enriched and socially isolated contexts while others use enriched and impoverished

conditions. The primary differences between the terms used for different environmental contexts are the number of rats housed together, amount of handling, and whether there are objects for the rats to manipulate (Renner & Rosenweig, 1987; Bardo & Dwoskin, 2004). The context designations that many researchers currently use are enriched condition (EC), impoverished condition (IC), and social condition (SC). EC rats are typically housed in large cages of twelve to fourteen rats. EC rats generally have a greater amount of living space per rat than IC rats. EC rats have objects in the cage that are changed daily to provide a novel environment, and the rats are handled on a daily basis. IC rats are housed in hanging wire cages, in which there is no enrichment, social contact, nor are the rats handled. SC rats are housed in pairs for social contact; however, the rats do not have toys for enrichment, nor are they handled. The SC group used today is the typical housing condition as defined by National Institute of Health (NIH) guidelines (National Research Council, 1996).

Much of the literature investigating the enrichment paradigm consists of researchers using EC and IC groups, however, what is used as a social condition varies, if a social condition is present at all. In the past either a social condition or a grouped condition was used. The social condition used in previous research is the same as the social condition used today, while in the grouped condition several rats are housed in an enriched condition cage without novel objects (Renner & Rosenzweig, 1987). In many instances the paired social condition group was not used because results were the same as for impoverished condition rats (Mirmiran, Van den Dungen, & Uylings, 1982). Welch, Brown, Welch, and Lin (1974) observed that social housing created neurological differences. However, it was later shown that while social housing may play a role in

some of the enrichment effects, it does not appear to be involved in the neurological characteristics developed from enrichment housing (Rosenzweig, Bennett, Hebert, & Morimoto, 1978).

Previous research has shown that neuroanatomical changes begin to occur after four days of rearing in EC or IC groups (Rosenzweig, & Bennett, 1978). Behavioral effects occur in rats housed in EC or IC groups from 16 to 45 days of age, and are not altered after 45 days of age. Thus, 30 days of rearing in an EC or IC group is sufficient to produce behavioral effects (Einon & Morgan, 1977). Significant differences in dopamine transporter (DAT) surface expression have been shown in EC compared with IC rats after 30 days of rearing (Zhu, Apparsundaram, Bardo, & Dwoskin, 2005). Based on this research, rats in the current study were reared in either EC, IC, or SC groups for 30 days.

There are several neurological and neurochemical changes in rats raised in EC compared with IC or SC groups. Diamond, Rosenzweig, and Krech (1965) found that IC rats have significantly larger skeletons and skulls compared to EC rats. It is assumed that this difference is due to the decreased activity in IC rats, and because non-socially housed rats consume more food (Bardo & Dwoskin, 2004). Interestingly, even though the skeletal structures of IC rats are larger than EC rats, the brains of IC rats are significantly smaller than those of EC rats (Renner & Rosenzweig, 1987). This difference in brain size correlates with changes in the neurons in the brain (Renner & Rosenzweig, 1987). Researchers have found that EC rats have more myelinated axons and fewer unmyelinated axons than IC rats in the corpus callosum (Kopcik, Juraska, & Washburne, 1986), but a greater number of unmylenated axons overall in the brain (Juraska & Meyer, 1986). EC rats also have a greater number of dendritic spines than IC rats in the occipital

cortex and temporal cortex (Volkmar & Greenough, 1972; Greenough, Volkmar, & Juraska, 1973). These are significant neurological findings because they suggest that EC rats have a greater number of synaptic connections (Renner & Rosenzweig, 1987).

In addition to the anatomical changes, there are also several neurochemical changes as a result of environmental enrichment. It has been shown that EC rats have greater levels of dopamine (DA) in the cortex compared to IC rats (Riege & Morimoto, 1970). Consistent with this observation, DA mediated behaviors are altered in EC rats that receive psychostimulant drugs (Bardo & Dwoskin, 2004). This has several implications as the mesolimbic DA pathway is the primary pathway involved in psychostimulant use (Badiani, Oates, Day, Watson, Akil, & Robinson, 1998), and it is responsible for the motivational and rewarding effects of psychostimulants (Bardo, Bowling, Rowlett, Manderscheid, Buxton, & Dwoskin, 1995; Koob, 1999). Additionally, the mesolimbic DA pathway is activated following exposure to a novel environment (Koob, 1999; Bardo & Dwoskin, 2004). Thus, it has been suggested that environmental rearing may play a large role in future psychostimulant use (Bardo et al., 1995).

Psychostimulants

As described above, the mesolimbic DA pathway is the primary pathway in psychostimulant use. The ventral tegmental area, nucleus accumbens (NAcc), caudate putamen, medial prefrontal cortex (mPFC), amygdala, and the connection between the ventral tegmental area and the basal forbrain are the primary components of the mesolimbic DA pathway (Koob, 1999). Researchers have found that psychostimulants such as amphetamine and cocaine primarily increase levels of DA in the caudate putamen and the NAcc. The DA release in these two areas produces the behavioral effects of

amphetamine (Wise & Bozarth, 1987). When 6-hydroxydopamine is used to lesion the mesolimbic dopamine system, self-administration of amphetamine decreases (Day et al., 2001; Koob, 1999). When the NAcc is lesioned using 6-hydroxydopamine, a decrease in amphetamine-induced locomotor activity results (Kelly, Seviour, & Iversen, 1975). Studies have shown that infusions of amphetamine or cocaine into the NAcc (Hoebel, Monaco, Hernaandez, Aulisi, Stanley, & Lenard, 1983), basolateral amygdala (BLA) (Ledford, Fuchs, & See, 2003), or the central nucleus of the amygdala (ACe) (Chevrette, Stellar, Hesse, & Markou, 2002) are rewarding. Infusions of the D1 agonist SCH23390 into the dorsolateral bed nucleus of the stria terminalis, NAcc, or the ACe, increases cocaine self-administration (Epping-Jordan, Markou, & Koob, 1998). This is interesting as it is hypothesized that the central-extended amygdala is continuous with the NAcc shell (Alheid & Heimer, 1988; Heimer, deOlmos, Alheid, & Zaborsky, 1991). However, when SCH23390 is infused into the BLA it attenuates cocaine self-administration (Epping-Jordan et al., 1998). When high doses of raclopride, a D2 agonist, are infused into the BLA cocaine self-administration is attenuated, but cocaine self-administration increases when raclopride is infused at low doses (Berglind, Case, Parker, Fuchs, & See, 2006).

Regions of the mesolimbic DA system associated with the rewarding effects of psychostimulants also express *c-fos* following amphetamine administration. *C-fos* expression in the ACe, BLA, dorsal caudate putamen, and ventral tegmental area increases following amphetamine administration (Day et al., 2001; Neisewander, Baker, Fuchs, Tran-Nguyen, Palmer, & Marshall, 2000). *C-fos* expression increases in the NAcc core and shell, and the BLA following exposure to cocaine self-administration

(Neiswander et al., 2000). However, Howes and colleagues (2000) found that rats administered cocaine had significantly greater *c-fos* expression in the ACe, NAcc shell, and core, but not in the BLA. These results clearly support the hypothesis that the ACe and BLA, in addition to the NAcc and caudate putamen, all play a role in mediating the effects of psychostimulants via the mesolimbic DA reward pathway.

Enrichment and Psychostimulants

Based on the previous research mentioned above, it is clear that psychostimulants influence behavior; however, one's environment also influences behavior, and this may be the key to determining how and to what degree subjects will respond when administered amphetamine. Researchers have found that when EC rats are given an acute amphetamine administration, they are more sensitive to the rewarding effects and experience greater behavioral effects compared to IC rats (Bardo et al., 1995). However, when EC rats receive repeated amphetamine administrations they exhibit less sensitization compared with IC rats (Bardo, Klebaur, Valone, & Deaton, 2001). A high amphetamine dose produces sensitization in both EC and IC rats, but a low amphetamine dose only produces sensitization in IC rats (Bardo et al., 1995). Following repeated amphetamine exposure EC rats also show greater levels of amphetamine-induced hyperactivity compared to IC rats; but this finding is dose dependent (Bowling & Bardo, 1994; Bardo et al., 1995). EC rats display greater amphetamine-induced hyperactivity compared with IC rats following an acute administration of a moderate dose of amphetamine (1.0 mg/kg), but there is not a significant difference in amphetamine induced hyperactivity in EC and IC rats following a low unit dose (0.1 or 0.3 mg/kg) (Bardo et al., 1995).

Consistent with the dose dependent effects of enrichment on amphetamine-induced hyperactivity, past research has revealed dose dependent differences in amphetamine self-administration in EC and IC rats. At high doses there are no differences in the amount of amphetamine EC and IC rats self-administer, but at low doses EC rats self-administer less amphetamine than IC rats (Bardo, Klebaur, Valone, & Deaton, 2001). It has also been shown that inactivating the ACe attenuates responding in IC rats at low doses of amphetamine (Cain, Stairs, Brown, & Bardo, 2005).

The ability of enrichment to alter responding to amphetamine may be associated with differences in DA function. It has been hypothesized that the decrease in psychostimulant induced motor activity in EC rats is due to an enrichment-induced decrease in DA receptors in the mPFC (Del Arco et al., 2007). In-vivo studies have shown lower levels of dopamine clearance and DAT surface expression in the mPFC in EC rats compared to IC rats (Zhu et al., 2005; Bardo & Dwoskin, 2004). EC rats also exhibit lower levels of activity compared with IC rats following administration of a DAT inhibitor (GBR12935) (Zhu, Green, Bardo, & Dwoskin, 2004). These studies provide evidence that DA plays a role in the behavioral effects following amphetamine exposure in EC and IC rats. Interestingly, there appears to be no differences in DA levels between EC and IC rats in the NAcc or striatum (Bardo et al., 1995).

C-fos and Psychostimulants

C-fos is a marker of neuronal activation that can be immunofluorescently tagged, thus, allowing researchers to identify neuronally activated regions of the brain. The *c-fos* protein is activated in response to novel stimuli and exposure to psychostimulants (Badiani et al., 1998). The *c-fos* protein is produced as the *c-fos* gene binds with the *c-*

jun gene to form a transcription factor, known as activating protein one (AP-1) (Kalthoff, 2001). AP-1 then binds to the promoter region of various genes, activating either transcription or phosphorylation, thus producing the *c-fos* protein. *C-fos* fluorescent tagging is then used to quantify levels of *c-fos* mRNA expression (Herrlich & Angel, 1994).

Psychostimulants produce widespread *c-fos* expression in the caudate putamen, NAcc, and olfactory tubercle, while localized *c-fos* expression is found in the striatum, septum, amygdala, neocortex, entorhinal cortex, NAcc, hypothalamus, periaqueductal gray matter, substantia nigra, and ventral tegmental area (Day et al., 2001; Graybiel, Moratalla, & Robertson, 1990; Umino, Nishikawa, & Takashi, 1995). The ACe has been shown to express *c-fos* following cocaine (Howes et al., 2000), methamphetamine (Umino et al., 1995), and amphetamine (Engber, Koury, Dennis, Miller, Contreras, & Bhat, 1998) exposure. The BLA also expresses *c-fos* following cocaine (Neisewander et al., 2000), methamphetamine (Umino et al., 1995), and amphetamine (Day et al., 2001) administration. Researchers have found that the greatest levels of *c-fos* expression occur when using high doses of psychostimulants. However, such high doses have been reported to decrease the amount of rearing on the active lever during acquisition of self administration (Howes et al., 2000). Although high doses of psychostimulants may assure *c-fos* expression, this implies that high levels of psychostimulants may not replicate effects of normal drug use.

The ACe and BLA, which are involved in reward, also express *c-fos* when rats are exposed to environmental novelty (Badiani, Camp, & Robinson, 1997), though results vary. When rats were exposed to environmental novelty following amphetamine (<3.0

mg/kg), levels of *c-fos* in the core and shell of the NAcc and in the caudate nucleus were greater than levels of *c-fos* for either amphetamine or novelty alone (Badiani et al., 1997). Rats that were exposed to environmental novelty following a 1.5 mg/kg dose of amphetamine showed greater levels of *c-fos* in the BLA than when administered amphetamine or exposed to novelty alone (Day et al., 2001). While exposure to novelty or amphetamine administration produces significant increases in *c-fos* expression in the ACe, *c-fos* expression is not as robust following exposure to novelty alone, or when novelty and amphetamine are paired (Day et al., 2001). Because *c-fos* expression in the BLA and NAcc is greater when amphetamine and novelty are paired, but this is not the case in the ACe, it is believed that novelty and amphetamine induce *c-fos* expression in different areas of the brain (Badiani et al., 1998; Day et al., 2001). Studies have shown that novelty alone induces *c-fos* expression in the cortex, septum, ACe, and BLA, but not the core or shell of the NAcc. However, amphetamine induces *c-fos* expression in all of the above areas except the shell of the NAcc (Badiani, et al., 1998; Day et al., 2001). Interestingly, DA neurons are activated in the NAcc and the mPFC when entering a new environment (Rebec, Grabner, Johnson, Pierce, & Bardo, 1997). The dopaminergic neurons in these areas are also critically involved in producing the rewarding effects of drugs (Koob, 1999). This implies that while the different areas of the brain may independently contribute to novelty and drug abuse, pathways such as the mesolimbic dopamine system may be working together (Rebec et al., 1997, Koob, 1999). Thus, it is believed that *c-fos* levels in the ACe and BLA, following acute amphetamine exposure, will vary with the environmental context in which rats were raised.

To understand the interaction between enrichment and psychostimulants, the present study examined the effects of a moderate dose of amphetamine on amphetamine-induced hyperactivity and *c-fos* expression in the ACe and BLA in EC, IC, and SC rats. It was hypothesized that EC rats would show greater levels of amphetamine induced hyperactivity following acute amphetamine exposure (1.0 mg/kg) compared with IC and SC rats. Using this moderate dose of amphetamine, it was also hypothesized that EC rats would display greater levels of *c-fos* expression in the ACe and BLA compared with IC and SC rats. If the above hypotheses were supported, it would demonstrate that the rearing environment influences amphetamine-induced hyperactivity and *c-fos* expression. Further, these findings would assist in determining whether the ACe and BLA contribute to the differential response to amphetamine across environmental conditions. If differences within the ACe and BLA were observed across environmental conditions, it would suggest that environmental rearing may cause neuronal changes that could possibly influence future drug use and abuse.

Method

Animals

Male Sprague-Dawley rats (Charles River, Portage, MI, USA) arrived in the laboratory at 21 days of age (n=48). Rats had ad libitum access to food and water, and were housed in an animal colony on a 12 hour light/dark cycle with lights on from 700 to 1900 hours. The animal colony was maintained at 22° C and humidity ranged from 30-45%. The procedure was approved by the Institutional Animal Care and Use Committee and Kansas State University, and complies with NIH guidelines (National Research Council, 1996).

Environmental Conditions

Upon arrival rats were placed into one of three environmental conditions (EC, IC, or SC) for the remainder of the experiment. EC rats (n=16) were housed in groups of 8 in large metal cages (60x120x45 cm) that were lined with paper pulp bedding. Fourteen plastic objects (children's toys and PVC pipe) were placed in each cage. Seven of these objects were changed daily, and all toys were changed twice weekly. EC rats were handled daily during toy changes. IC (n=16) rats were housed individually in hanging metal cages (17x24x20 cm) that had wire mesh front and bottom, and solid sides. IC rats were not handled during rearing (21-51 days of age). SC rats (n=16) were housed in pairs in standard plastic shoebox cages (20x43x20 cm). SC cages had paper pulp bedding and wire tops, and SC rats were handled weekly during scheduled cage changes.

Testing Apparatus

Locomotor activity was measured by recording photobeam interruptions in a locomotor chamber measuring 40.64 cm x 40.64 cm x 40.64 cm (Coulbourn Instruments, TruScan 2.01). The chambers had plexiglass walls and a stainless steel floor covered with pine-chip bedding. The photobeam sensor ring consisted of a 16 (X axis) by 16 (Y axis) photocell array. The photocells in each axis were spaced 2.54 cm apart (center to center). Throughout the session a 70 decibel white noise was generated to eliminate background noise.

Drug

D-amphetamine was dissolved in 0.9% saline (1.0 mg/ml) and injected subcutaneously. D-amphetamine was obtained from Sigma Aldrich, Dallas, TX, USA.

Procedure

Locomotor: Amphetamine-Induced Hyperactivity

On days 51- 56 rats were administered either a moderate dose of amphetamine (1.0 mg/kg, sc; EC=8, IC=8, SC=8) or saline (EC=8, IC=8, SC=8) immediately prior to being placed in the locomotor chambers for 60-min, during which horizontal locomotor movement was recorded. Each day 8 rats were tested in a balanced order according to treatment and environmental condition. Immediately following completion of the 60-min locomotor session the rats were deeply anesthetized by pentobarbital (390 mg/mL sodium pentobarbital & 0.29 mL/mL ethyl alchohol) injection (i.p. 40 mg/kg) and brains were fixed via transcardial perfusion with a heparinized saline rinse, followed by a 4% paraformaldehyde fix for 30-min. Brains were removed and placed in a sucrose cryoprotectant, and stored at room temperature.

Immunohistochemistry

A general avidin-biotin-peroxidase complex (ABC) procedure was used (Vectastain kit) as previously described (Huang & Weiss, 1999; Weiss & Chowdhury, 1998). The brains of thirty-six rats were processed (6 EC, 6 IC, 6 SC per treatment). The remaining brains were saved in the event that additional brains were required. The size of groups was determined using previous *c-fos* literature (Badiani et al., 1998; Crombag, Jedynak, Redmond, Robinson, & Hope, 2002). Frozen brains were sliced into serial transverse sections (40 μm) using a Reichert-Jung-Leica cryostat and sections containing the ACe (anterior-posterior, -2.3 mm; medial-lateral, $\pm$ 4.2 mm; dorsal-ventral, -6.5 mm) and BLA (anterior-posterior, -2.8 mm; medial-lateral, $\pm$5.0 mm; dorsal-ventral, -8.4 mm) (Paxinos, & Watson 1998) were collected. The sections were then incubated in a

blocking solution of 5% normal goat serum diluted in 0.2% Triton X-100 in phosphate buffered saline (PBS-TX, 50 mM mixed phosphate buffer, 150 mM NaCl, pH 7.4) for 30-min; PBS-TX was used for all subsequent dilution and washing steps. Free floating tissue sections were reacted with rabbit anti-*c-fos* primary antibody (1:500, Santa Cruz; 12- hr at room temp), biotinylated secondary antibody (3-hr at room temperature), and avidin-biotin-peroxidase complete (3-hr at room temperature), respectively. The *c-fos* immunoreactive neurons were colored with 0.01% H_2O_2 and 0.05% diaminobenzidine. The sections were mounted on gelatinized slides and coverslipped with DPX. Brightfield illumination was used to examine the cells visualized with diaminobenzidine.

Data Collection

Cells positively stained for *c-fos* using diaminobenzidine had a dark brown nucleus with an unstained cytoplasm. Three sections that were evenly spaced anterior to dorsal were selected from each brain, and the number of *c-fos* cells were quantified by hand counting the cells in the selected areas. The mean amount of *c-fos* expression was calculated from the 3 selected slices for each brain, and used for statistical analysis. The tissue was coded after fixation and processed blind to the experimental conditions to prevent bias. At least 33% of the slices were coded by a second blind observer and both observers were in agreement. The results are expressed as mean and standard errors. The tissue was processed in a single batch to prevent batch to batch differences in immunocytochemical staining.

Statistical Analysis

Horizontal locomotor movement was analyzed using a 2 X 3 between subjects analysis of variance (ANOVA). Quantified *c-fos* expression was analyzed using a 2 X 3

between subjects ANOVA for each brain area. For both between subjects ANOVAs environmental condition and treatment group served as the between subjects factors. Multiple comparisons were used to probe any significant interactions. Alpha was deemed significant at $p<.05$.

Hierarchical regression analysis was performed to investigate whether amphetamine-induced hyperactivity predicted *c-fos* expression. *C-fos* expression in the ACe or BLA served as the dependent variable, and locomotor activity, environmental context, and treatment served as independent variables. Continuous variables were standardized prior to performing the hierarchical regression. For both hierarchical regressions, standardized distance traveled was entered in the first step, environmental condition was added in the second step, and injection was entered as the third step.

Results

Locomotor Activity: Amphetamine-Induced Hyperactivity

The total distance traveled (cm) was analyzed using a 2 X 3 between subjects ANOVA where environmental condition and treatment served as between subjects factors. Due to technical errors, locomotor data could not be obtained for 2 SC rats and 1 IC rat, thus, the mean from the appropriate treatment groups of SC and IC rats was calculated and substituted. Results showed a main effect of treatment, $F(1,31)=54.88$, $p<0.001$. There was also a main effect of environmental condition, $F(2,31)=16.99$, $p<0.001$, and a significant interaction between treatment and environmental condition, $F(2,31)=16.99$, $p<0.001$. Simple effects revealed that SC rats had significantly greater levels of locomotor activity compared to EC, $F(1,2)=18.19$, $p<0.05$, and IC, $F(1,2)=25.35$, $p<0.05$, rats regardless of treatment. Additionally, EC rats treated with

amphetamine had significantly greater locomotor activity compared with EC rats treated with saline, $F(1,2)=24.54$, $p<0.05$ (Figure 1).

Due to differences in significance findings in the current study compared to previous literature, 2-tailed independent samples t-tests were conducted. Results revealed that SC rats administered amphetamine, $t(10)=3.51$, $p<0.01$, or saline, $t(10)=3.93$, $p<0.01$, had significantly greater locomotor activity compared to IC rats administered amphetamine or saline. SC rats administered amphetamine, $t(10)=2.59$, $p<0.03$, or saline, $t(10)=3.67$, $p<0.01$, also had significantly greater locomotor activity than EC rats. Additionally, treatment had a significant impact in EC, $t(10)=5.12$, $p<0.001$, IC, $t(10)=3.95$, $p<0.01$, and SC rats, $t(10)=3.29$, $p<0.01$, as rats that received amphetamine had significantly greater locomotor activity compared to rats administered saline.

C-fos Expression

C-fos expression in the ACe and BLA was analyzed using a 2 X 3 between subjects ANOVA in which environmental condition and treatment served as between subjects factors. Results showed no main effects or interactions of *c-fos* expression in the ACe or BLA. Simple effects analyses of *c-fos* expression within the ACe revealed no significant differences in *c-fos* expression (Figure 2).

Simple effects analyses of *c-fos* expression within the BLA revealed that IC and SC rats had significantly greater levels of *c-fos* expression in the BLA compared to EC rats, independent of treatment, $F(1,2)=38.43$, $p<0.05$, $F(1,2)=42.05$, $p<.05$. IC saline rats had significantly greater levels of *c-fos* expression compared to EC saline rats, $F(1,2)=25.465$, $p<0.05$. SC rats treated with amphetamine had significantly greater

levels of *c-fos* expression compared to EC amphetamine rats, $F(1,2)=24.028$, $p<0.05$ (Figure 3).

Locomotor Activity & c-fos expression

A hierarchical regression analysis was performed to investigate whether amphetamine-induced hyperactivity predicted *c-fos* expression. *C-fos* expression in the ACe or BLA served as the dependent variable, and locomotor activity, environmental condition, and treatment served as independent variables. Results revealed that neither locomotor activity, environmental condition, nor treatment predicted *c-fos* expression.

Discussion

The results of the current study revealed that environmental enrichment significantly alters *c-fos* expression in the BLA, but not in the ACe. Environmental enrichment also appears to significantly influence amphetamine-induced locomotor activity. These results suggest that differential rearing alters neural activity in the amygdala and alters behavior.

Previous literature has observed that EC rats have greater amphetamine-induced hyperactivity compared to IC rats at a 1.0 mg/kg dose (Bowling & Bardo, 1994; Bardo et al., 1995). Therefore, it was hypothesized that EC rats would show greater levels of amphetamine-induced hyperactivity compared with IC and SC rats. In the current study SC amphetamine and saline rats had significantly greater levels of hyperactivity compared to EC and IC amphetamine and saline rats. These results confirm previous research in our laboratory that observed significantly greater hyperactivity in SC rats compared to EC and IC rats when a 1.0 mg/kg dose of amphetamine is administered (Ha, Kabriel, Steiner, Parrish, & Cain, 2006). When rats are administered a 0.3 mg/kg dose repeatedly, SC rats initially show greater levels of hyperactivity than IC rats. However,

with repeated amphetamine treatment at a 0.3 mg/kg dose SC and IC rats show similar levels of hyperactivity for the majority of the sessions (Neises, Pittenger, Gill, & Cain, 2006). Bowling and Bardo (1994) observed that with repeated amphetamine administrations SC rats have less locomotor activity than EC rats when administered a .5 mg/kg or 2.0 mg/kg. Based on these results the differences in enrichment locomotor activity appear to be dependent upon dose and frequency, thus, the current results are in accordance with previous studies using a 1.0 mg/kg dose.

The current study did not find any significant differences in *c-fos* expression in the ACe. It was hypothesized that EC rats would show significantly greater *c-fos* expression compared to IC and SC rats. This hypothesis was based on the expectation that EC rats would have greater amphetamine-induced hyperactivity than IC rats. In addition, it was hypothesized that rats administered amphetamine would show significantly greater *c-fos* expression compared to rats administered saline. This hypothesis was based on past research that has shown that amphetamine induces *c-fos* expression in the ACe (Engeber, Koury, Dennis, Miller, Contreras, & Bhat, 1998; Day et al., 2001).

Differences between ACe *c-fos* expression in the current study and previous literature may be due to the effects of environmental rearing on novelty. In the current study environmentally enriched rats were exposed to novelty daily throughout rearing which has been shown to induce neurological changes (Renner & Rosenzweig, 1987), whereas previous studies did not expose rats to novelty during rearing. Results of the current study do not support the role of the ACe in response to novelty, although the literature appears to be inconsistent. *C-fos* expression has been shown to increase in the

ACe following exposure to novelty (Day et al., 2001). In contrast, Ostrander et al. (2003) showed that novelty following exposure to a cocaine associated cue attenuated *c-fos* expression. The role of the ACe in novelty detection appears to be species specific as Montag-Sallaz, Welzl, Kuhl, Montag, & Schachner (1999) found that mice have increased *c-fos* expression in the amygdala following exposure to novelty. In contrast, Moses, Sutherland, & McDonald (2002) showed that the ACe is not essential for novelty detection in rats. Interestingly in humans and primates the amygdala does appear to play an essential role in novelty detection. It has been shown that there are individual neurons in the ACe that signal novelty detection in learning tasks (Wilson & Rolls, 1993; Rutishauser, Mamelak, & Schuman, 2006). Neuronal activity in the amygdala is also associated with pupil size during novelty detection (Demos, Kelley, Ryan, Davis, & Whalen, in press; Whalen et al., 2004). In humans, the amygdala is hypothesized to improve novelty detection by activating cholinergic neurons that release acetylcholine. In turn the acetylcholine decreases the sensory system's neuronal thresholds (Whalen, 1998; Davis & Whalen; 2001). Thus, the amygdala allows humans to be more aware of novel and ambiguous cues. However, because these novelty-induced changes within the amygdala appear to be species specific, and it is unknown whether this cholinergic pathway is active during novelty in rats, we currently can not determine the role of the ACe in novelty detection in rats. Results of the current study would not lend support to novelty detection-induced ACe activation as EC rats had the least amount of activation in the ACe compared to IC and SC rats.

Although few studies have investigated the role of the BLA in novelty detection, results of the current study suggest that the BLA is involved in novelty. In the current

study it was hypothesized that EC rats would show significantly greater *c-fos* expression in the BLA compared to IC and SC rats. Interestingly, results revealed that regardless of treatment, IC and SC rats had significantly greater *c-fos* expression compared to EC rats.

To date no literature has investigated *c-fos* expression in differentially reared rats, but past studies have found that socially housed mice have greater *c-fos* expression of the immediate early gene zif-268 in the NAcc shell and core compared to enriched mice following cocaine (Solinas, Thiriet, Rawas, Lardeux, & Jaber, 2008). This is significant as the NAcc is part of the mesolimbic DA system. In addition to the neuronal changes in response to novelty, there are also behavioral changes (Renner & Rosenzweig, 1987; Bardo & Dwoskin, 2004). EC rats display less activity than IC rats in an inescapable novel environment (Bowling, Rowlett, & Bardo, 1993). Novelty appears to be less stressful for EC rats compared to IC rats (Bowling et al., 1993), and EC rats lose interest in novel stimuli more quickly (Zimmermann, Stauffacher, Langhans, & Würbel, 2001). Because EC rats are exposed to novelty daily, and do not appear to have as great of a behavioral response to novelty as IC rats, it is reasonable that EC rats would have a decrease, or no change in *c-fos* expression in the BLA and ACe.

Results of *c-fos* expression in the ACe and BLA in the current study are consistent with previous findings showing increased *c-fos* expression in the BLA when exposed to novelty but only minimal increases, or attenuation of *c-fos* expression in the ACe, when exposed to novelty (Ostrander et al., 2003; Day et al., 2001). These differences in *c-fos* expression in the ACe and BLA could be due to differences in dopaminergic neurons. Past research has revealed that exposure to novel stimuli produces increased levels of DA in the mPFC (Beaufour, Le Bihan, Hamon, & Thiebot, 2001; Feentra & Botterblom,

1996). DA neurons in the mPFC are also activated following psychostimulant administration, suggesting that the mesolimbic DA system may play a role in novelty and psychostimulant use (Hertel, Mathé, Nomikos, Iurlo, Mathé, & Svensson, 1995). Differences in the DA system are also apparent in EC and IC rats as EC rats have decreased DAT surface expression in the mPFC compared with IC rats (Zhu, Green, Bardo, & Dwoskin, 2004; Zhu et al., 2005). These changes in DA may be associated with levels of *c-fos* expression as Umino et al. (1995) hypothesized that DA receptors mediate psychostimulant induced *c-fos* expression. Thus, differing *c-fos* expression in the ACe and BLA may be due to rearing-induced dopaminergic differences in the two areas.

Previous research has found differences in the dopamine receptors activated by novelty or amphetamine. Badiani, Oates, Day, Watson, Akil, & Robinson, (1999) reported that, when rats are exposed to amphetamine alone, there is a significant increase in *c-fos* expression in D1 receptors in the caudate. It is only when amphetamine is paired with environmental novelty that both D1 and D2 receptors are activated. Additionally, D2 receptors continue to be expressed following inactivation of the medial forebrain bundle, suggesting that different dopamnergic systems are at work during novelty and amphetamine exposure (Badiani et al., 1999; Uslaner et al., 2001). Interestingly, studies looking at DA receptors in environmentally enriched rats are varied. Del Arco and colleagues (2007) showed a decrease in D1 receptors in the mPFC of EC rats. Bardo and Hammer (1991) found no differences between D1 and D2 receptor levels in the NAcc or mesolimbic DA systems of EC and IC rats. However, EC rats do have decreased DA uptake, metabolism, and DAT expression in the mPFC compared to IC rats (Zhu et al.,

2004; Bardo and Dwoskin, 2004; Zhu et al., 2005). Thus, differences in *c-fos* expression in the ACe and BLA may be accounted for by different DA receptors, and which receptors are activated by amphetamine and novelty.

The varied results regarding DA receptors and *c-fos* expression following amphetamine may be due to differences in the dose of amphetamine used. Levels of *c-fos* expression in the current study may differ from previous studies as a much lower amphetamine dose was used. In previous studies rats received either a 2.0 mg/kg or 5.0 mg/kg intraperitoneal injection (Day et al., 2001; Engber et al., 1998), while the current study used a lower 1.0 mg/kg subcutaneous injection. Despite the finding that levels of *c-fos* expression in the ACe and BLA appear to be greater following high doses of amphetamine, the current study used a moderate dose of amphetamine. A moderate rather than a high dose of amphetamine was used in the current study, as previous research has shown that the differences between EC and IC rats in amphetamine self-administration are not present at high unit doses (Bardo et al., 2001). In the future, a low dose (0.3 mg/kg), a moderate dose (1.0 mg/kg), and a high dose (2.0 mg/kg) could be used to determine whether enrichment and amphetamine influence *c-fos* expression dose dependently.

In addition to the dose of amphetamine used, the number of times amphetamine is administered may also influence *c-fos* expression. Future studies will measure *c-fos* expression following repeated injections of amphetamine, revealing the role of novelty of the locomotor chambers in *c-fos* expression. Using repeated administrations rather than an acute injection will also allow the investigation of whether differences in sensitization between EC and IC rats are reflected in activation of particular brain regions. Repeated

amphetamine injections have been found to attenuate locomotor sensitization in EC rats compared with IC rats (Bardo et al., 1995). Differences in *c-fos* expression are also dependent on the area being investigated. In the current study only the ACe and BLA were analyzed. However, slices were taken from the NAcc and mPFC as they play a critical role in the mesolimbic DA pathway (Koob, 1999), and they are regions that have previously shown differences between EC and IC rats (Renner & Rosenzweig, 1987). Slices from the NAcc and mPFC are currently being processed to determine *c-fos* expression following an acute amphetamine exposure. Additional sections were also taken from the parastriatal nucleus to serve as a control site, and these will also be analyzed at a later date.

The current study demonstrated that environmental rearing significantly influences locomotor activity and *c-fos* expression in the BLA, while it does not significantly influence *c-fos* expression in the ACe. These results suggest that differential rearing environments neurochemically influence one's response to psychostimulants. The ability of differential rearing to alter *c-fos* expression following psychostimulant administration may contribute to the ability of enrichment to decrease self-administration of psychostimulants.

Figures

Figure 1. Mean locomotor distance in cm, during the 1-hr locomotor session comparing EC, IC, and SC rats that received a 1.0 mg/kg dose of amphetamine or saline. An asterisk (*) represents a significant difference between EC amphetamine and EC saline rats, $p<0.05$.

Figure 2. Mean amount of *c-fos* expression in the ACe for EC, IC, and SC rats that were administered either amphetamine or saline.

Figure 3. Mean amount of c-fos expression in the BLA for EC, IC, and SC rats that were administered either amphetamine or saline. A carrot (^) represents a significant difference between EC and SC amphetamine rats, $p<0.05$. An asterisk (*) represents a significant difference between EC and IC saline rats, $p<0.05$.

Figure 1

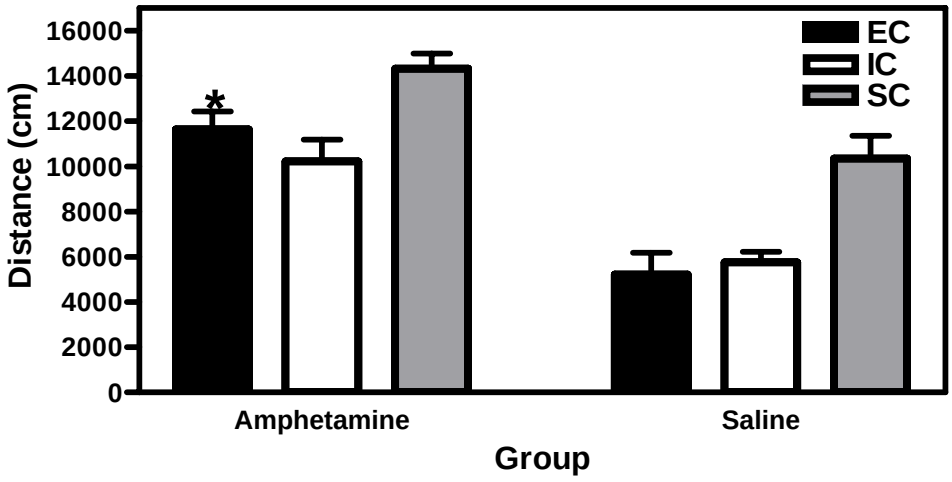

16000
14000
12000
10000
8000
6000
4000
2000
0
Distance (cm)
*
EC
IC
SC
Amphetamine
Saline
Group

Figure 2

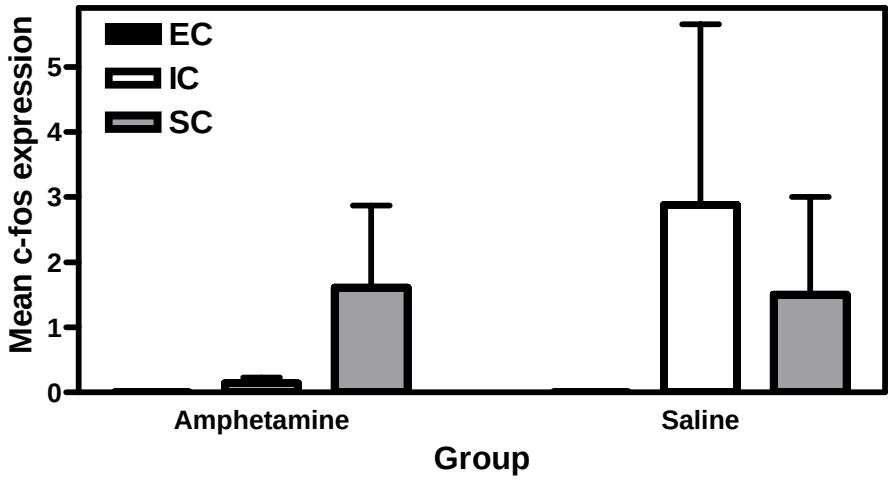
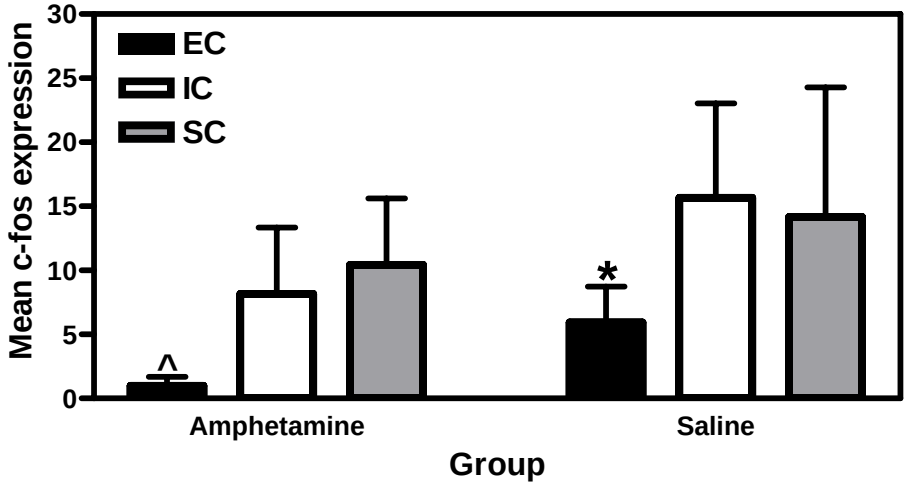

EC
IC
SC
Mean c-fos expression
0
1
2
3
4
5
Amphetamine
Saline
Group

Figure 3


Mean c-fos expression
30
25
20
15
10
5
0
EC
IC
SC
^
*
Amphetamine
Saline
Group

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