Enrichment Influences Social Preference Behavior in Older Female and Male Adolescent Rats

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Abstract

Rats engage in exploratory and novelty-seeking behaviors in adolescence to gain knowledge about their environment. Environmental enrichment (EE) provides a setting in which rats can explore and experience novelty through aspects of the physical environment and interacting with conspecifics. In this study, the effects of EE on the behavior of adolescent rats during a social preference task was investigated, as well as EE's effects on neural activation in the basolateral amygdala (BLA) and *cornu ammonis* 2 (CA2) region of the hippocampus. Adolescent Long-Evans rats (n=12) experienced EE between postnatal days (pnd) 23 and 48; age-matched controls (n=18) experienced a non-enriched home cage. On pnd 49, a two-trial social preference task occurred in a walled open field. Proportion of time spent and proportion of contact initiated with the novel rat was measured. After the task, brain tissue was processed to identify neural activity in the BLA and CA2. A significant interaction of EE and sex on proportion of time spent with the novel rat was found; no-EE males displayed a larger proportion than no-EE females (p = .007). A significant interaction of EE and sex on proportion of contact initiations was also observed; EE males showed a larger proportion than EE females (p = .004). Histology indicated 60% less neural activation in the BLA and CA2 of EE rats (p = .001). While behavioral and neural data indicate that EE decreases rats' responses to novelty, maturation differences between sexes impacted behavior.

Keywords: social preference, adolescence, novelty preference

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Adolescence is a time of exploration and rapid growth in many species, including humans. This increased propensity for exploring and learning about one's environment during this period of life can manifest in behaviors that are risky in nature. Whether in humans or rats, this risk-taking behavior is a transient phase of behavioral decision-making that is adolescent-specific. In humans, risky behaviors include experimentation with drugs, sexual promiscuity, and getting tattoos or piercings, among other activities. In rats, risky behaviors can be measured through a few different tasks carried out in the controlled setting of the laboratory.

One task that can be used to observe risk-taking behaviors is a social preference task, which examines how the rat of interest chooses to interact with a rat familiar to them and a rat unfamiliar to them when placed in a situation where there is an equal opportunity to interact with both. In this case, preferential behavior towards a novel rat would be considered risky, as the rat of interest is choosing to interact with something unfamiliar, thus inherently more dangerous. The use of rats as a model for studying behavior is widely supported by research, especially regarding the period of adolescence, as monitoring and controlling facets of a rat's life such as development, social interactions, and environment is relatively easy (Simon, Gregory, Wood, & Moghaddam, 2013).

Environmental enrichment has been demonstrated to influence risky behaviors in rats in a few significant ways as well as produce changes in the brain related to plasticity and growth (Simpson & Kelly, 2011). Environmental enrichment involves enriching a rat both socially, through interaction with conspecifics, and physically, through the features of the enrichment cage. The effects of environmental enrichment on adolescent rats performing a social preference task was examined in this study, and how enriched adolescents behave differently than

unenriched adolescents as a task is performed was observed. Brain differences present between groups, specifically regarding neural activation in the basolateral amygdala and the *cornu ammonis* 2 (CA2) region of the hippocampus was also examined. It was of interest if enriched rats, when given the choice between interacting with a novel rat or a familiar rat in a social preference task, would display less overall contact with the novel rat than unenriched control rats. In addition, the differences in neural activation between enriched and unenriched rats in brain areas such as the amygdala were investigated. These things were of interest because of the way environmental enrichment has been suggested to change novelty-preference and social play behaviors (Simpson & Kelly, 2011; Siviy & Panksepp, 2011), as well as influence activation in brain regions such as the amygdala and the CA2 region of the hippocampus that act as neural correlates for emotion and memory related to these behaviors (Simpson & Kelly, 2011; Tanti, Rainer, Minier, Surget, & Belzung, 2012).

Adolescence

In rats, a generally accepted definition of adolescence quantifies it as the period of time from weaning at postnatal day (pnd) 21 to around pnd 59, which represents later adolescence (Lynn & Brown, 2009). Adolescence is a time of exploration in rats, during which young rats gain valuable life skills through learning about their environment, and risky behaviors are involved in this exploration and learning period, as novelty seeking is a facet of this process (Lynn & Brown, 2009). Previous research has demonstrated that both humans and rats alike show more impulsivity and higher sensation and novelty seeking behaviors in adolescence than in younger or older age groups (Stansfield & Kirstein, 2005). A few hypotheses have been suggested to account for this difference between age groups and how it can affect behavior. Some of these include adolescents possessing a capacity to see the possible risks or outcomes of

certain behaviors that is not yet fully developed, adolescents' cognitive processes differing from adults' regarding weighing the risks of a behavior against its benefits, and adolescents processing emotions related to risk-taking in a unique way (Stansfield & Kirstein, 2005; Sturman & Moghaddam, 2011). Others have attributed these differences between adolescence and other age groups to a hypersensitivity to stressful events during adolescence (Burke & Miczek, 2015).

Adolescence is also a time of great changes in the brain. In humans, brain changes in adolescence have been observed in the mesolimbic system and areas like the striatum, insula, and amygdala (Blakemore & Choudhury, 2006). There is a loss of gray matter in some areas, accompanied by an increase in white matter in cortical and subcortical areas. Other findings have indicated changes in activity in structures related to emotion (including the amygdala) during this period (Ashokan, Hedge, & Mitra, 2016; Koe, Ashokan, & Mitra, 2016).

Sturman and Moghaddam (2011) suggested that, when it comes to risk-taking behaviors in adolescence, the balance between three nodes in the brain is important. The first of these is the detection node, including areas in the occipital cortex and temporal cortex, and it is involved in recognizing whether a stimulus includes social information. The second node is the affective node, which includes the amygdala; its role is to take the information from the detection node and give emotional context to a stimulus. The third node is the cognitive-regulatory node, which consists of areas of the prefrontal cortex. This node takes the information from the detection and affective nodes and applies higher-order processing, such as the formulation of goal-directed behavior. Sturman & Moghaddam (2011) argue that, during adolescence, these nodes change in sensitivity and how they communicate with each other to strengthen the emotional experience of social interaction, which would influence the way adolescents make decisions.

Social Interaction

Rats are known to be very social animals. Behaviors such as play fighting that rats engage in prior to adulthood have been suggested to be essential to the process of developing normal social behaviors as adults, such as social flexibility, communication, and group cohesion (Burke & Miczek, 2015; Palagi et al., 2016; Trezza, Baarendse, & Vanderschuren, 2010). Social play differs between adolescents and adults. Play in adolescence involves one rat pouncing on another to access the nape of its neck, and the rat who is pounced upon will most often rotate onto its back, resulting in pinning. In adults, pinning is not as common (Palagi et al., 2016; Siviy & Panksepp, 2011; Trezza et al., 2010). In addition, social play has been demonstrated to be a rewarding process for animals. This is supported in part by rats emitting high frequency vocalizations while engaging in social play; these sounds are also emitted during behaviors such as sex that are known to be pleasurable (Palagi et al., 2016; Panksepp, 2011; Siviy & Panksepp, 2011; Trezza et al., 2010). Siviy & Panksepp (2011) indicated the effects of sex and familiarity on play in adolescent rats, with total time engaged in play remaining high for both sexes when playing with an unfamiliar rat, but males initiating more play with familiar rats and females initiating more play with unfamiliar rats.

The tendency towards impulsivity and riskiness in adolescence indicated in Lynn and Brown (2009) can be easily extrapolated to social interaction with conspecifics. Ferguson, Young, and Insel (2002) found that social recognition in rats plays an important role in survival as well as other chief aspects of life, such as reproduction. Social recognition is associated with social play in rats, as the more one rat plays with another, the more familiar and recognizable the rats become to one another. In other words, rats can become familiar through play. When a rat has the opportunity to choose to interact with a novel or a familiar conspecific in a setting

including both, it would be considered risky to spend more time interacting with the novel conspecific than the familiar one.

With social play in adolescence comes a maturation of the prefrontal cortex due to a number of factors, including positive emotions associated with play (Van Kerkhof, Damsteegt, Trezza, Voorn, & Vanderschuren, 2013); this maturation helps develop executive control functionality, such as the regulation of impulsivity (Palagi et al., 2016). With regard to the pleasurable aspect of social play, endogenous opioids are known to play a key role, particularly in just simply enjoying the act. The dopamine system is implicit in the rewarding aspect of play and is thought to take part in its modulation. The cannabinoid and noradrenergic systems also have been demonstrated to influence social play when examined in drug studies (Panksepp, 2011; Siviy & Panksepp, 2011; Trezza et al., 2010). Palagi et al. (2016) and Siviy and Panksepp (2011) discuss how subcortical areas, such as the limbic system, are essential to play, and cortical systems play more of a role in the regulation of these behaviors. Implications of amygdala involvement emerge when implicating amygdala lesions in early adolescence—these decreased play while not affecting other social behaviors in rats. In addition, an increase in c-*fos*, a neural activity marker, has been observed in the amygdala of hamsters following social play (Siviy & Panksepp, 2011).

When rats engage in social interaction, certain processes occur in the brain that facilitate the connection between memory and social recognition. Ferguson et al. (2002) discuss how both learning and memory come into play when interacting with conspecifics. When rats play and gain recognition for each other, each forms a memory for the other, and behavioral choices are made based on prior knowledge. This is exemplified through changing levels of vasopressin and oxytocin in systems involved in different aspects of learning and memory, play, and more

complex social behaviors. The amygdala is also implicated in social behaviors and social memory, as it contains an area that serves as a mediator for oxytocin's impact on social memory (Ferguson et al., 2002). The CA2 region of the hippocampus has been implicated in the acquisition and strengthening of social memories through research involving vasopressin signaling (Smith et al., 2016). Hitti & Siegelbaum (2014) suggested the CA2 as critical place through which animals process social memory. With the inactivation of pyramidal neurons in the CA2 region, mice displayed a loss of social memory, resulting in an inability to remember conspecifics. Thus, it would be expected for neurons in these regions to be active during tasks involving social recognition, such as social preference tasks.

Environmental Enrichment

Environmental enrichment (EE) with regard to rats involves allowing predetermined groups to interact with conspecifics, ramps, platforms, and toys in a novel enrichment cage that is separate from rats' home cages for a set amount of time per day. Physical enrichment is a term for the material features of the enrichment cage, like toys, that can be enriching for rats, while social enrichment relates to time and interaction with conspecifics that able to occur in enrichment cages (Simpson & Kelly, 2011). Donald Hebb was one of the first to identify the possible changes EE could produce in rats and how these changes might affect the outcomes of research studies; he first observed this in 1947 after allowing some of his lab rats to play with his children at home and finding that they subsequently performed better on tasks run in his lab (Hebb, 1947). To further support for enriching at younger ages, Forgays and Forgays (1951) found that when rats were enriched early in life, better performance on problem-solving tasks was observed later in life. Due to findings such as these, it is consistent to start EE at pnd 21 to 23 (Simpson & Kelly, 2011).

EE has been demonstrated to produce various effects on behavior and cognitive processes. Enriched rats have shown differences in learning and memory tasks, engaged in more social play, displayed quicker adaptation to novelty, and shown freer exploration of novel environments when compared to rats housed in standard group housing (Simpson & Kelly, 2011). Will et al. (1986) found that EE affected performance on a learning task in adolescent rats with hippocampal lesions, preference for a novel environment in intact adolescent rats, and object novelty preference in both lesioned adolescent rats and intact adolescent rats. The majority of the research examining the effects of EE has looked at novelty preference in the context of interaction with objects (e.g., Cobb, 2015). As EE itself involves both interaction with objects and other animals, this novelty preference effect seen with objects is thought to carry over to social preference, where novelty is represented by an unfamiliar rat.

The brains of rats that have experienced EE have been shown to respond differently in a number of ways, including in response to novelty. Mandolesi et al. (2008) found that, after basal forebrain lesion, EE produced positive effects on cognitive performance. In addition, changes in hormone levels and nucleotide levels have been observed with EE (Simpson & Kelly, 2011; Will et al., 1986). EE has been shown to enhance cortical growth and development through mechanisms such as neurogenesis and dendritic branching regardless of the animal's age, as well as play a protective role in drug use studies (Simpson & Kelly, 2011).

EE has been demonstrated to reduce negative emotional responses, such as anxiety, in rats, which researchers have observed through neural changes in the amygdala (Ashokan, Hegde, & Mitra, 2016; Koe, Ashokan, & Mitra, 2016; Okuda et al., 2009). Okuda et al. (2009) found that EE increased the number of progenitor cells in the amygdala of mice, as well as increased cell differentiation and repressed cell death. Koe, Ashokan, and Mitra (2016) examined the

effects of one short episode of enrichment in adulthood on the BLA of rats that had been separated from their dams repeatedly before weaning. Maternal separation in the unenriched group resulted in an enlargement of the BLA that lasted into adulthood, while the BLA of rats in the EE condition displayed a sort of remodeling in which the BLA was able to return to a normal size and state. Similarly, Ashokan, Hegde, and Mitra (2016) reported that a short bout of EE after stress resulted in a structural renormalization of the BLA, and rats that experienced stress but not enrichment showed higher levels of brain-derived neurotropic growth factor (BDNF), which indicates a stress-induced increase in cell size and number of dendrites in the BLA. These studies indicate the ameliorative effect of EE on the maladaptive responses in the body to stress and anxiety.

In the hippocampus, exposure to EE results in plasticity changes through long-term potentiation (LTP), including increased neurogenesis, dendritic branching, synapse formation, and cell size. Higher levels of neurotransmitters like noradrenaline and glutamate have also been observed here, which aids in plasticity changes (Simpson & Kelly, 2011; Stein, O'Dell, Funatsu, Zorumski, & Izumi, 2016). Tanti et al. (2012) found that, in the area of the hippocampus that contains CA2, EE increased neurogenesis and cell proliferation, as well as improved performance on a reference memory task and lessened anxiety-related behaviors.

Current Study

A social preference task is an effective way to examine how EE might affect the propensity towards risky behaviors seen in adolescent rats. EE has been shown to influence social interaction between rats; enriched rats show an increase in social play coupled with quicker adaptation to novelty and a tendency to explore a new environment more thoroughly (Simpson & Kelly, 2011). Tasks that allow researchers to measure how enriched versus

unenriched rats respond to novelty as well as examine social recognition would be ideal for identifying possible EE effects on social preference. Thor, Harrison, and Schneider (1988) used adolescent rats as stimuli for adult rats in a social preference task and found that adult rats preferred the unfamiliar rat, even after a long exposure to the familiar rat prior to the task. This use of adolescents as stimulus rats and adults as the subjects of interest is traditional and consistent with most of the literature in this area (Siviy & Panksepp, 2011). Utilizing age-matched adolescents as stimuli and as the subject of interest, as the current study does, is uncommon in the literature. Some studies look not only at time spent with novel or familiar rats but behavioral factors such as grooming, rearing, and nose pokes (Patterson, 2015; Thor et al., 1988), which give insight into an animal's investigation preferences, such as their attempts to contact or their willingness to be close to another animal. Though these parameters were not measured in this study, they are encompassed by the larger interest in social recognition present in carrying out social preference tasks. By putting animals together in an open field and letting them play, researchers facilitate recognition and the formation of social memory between the animals, and this is easily measured by observing the time animals spend together as well as how often they are initiating contact with one another.

The social preference task used in this study differs from what has been utilized in the majority of research in this area such that adolescent rats were used as both the subject of interest and the stimuli during the task. It is hoped that by utilizing exclusively adolescents in this social preference study, the paucity of research regarding the effects of EE on adolescent novelty-seeking behaviors can be attenuated to some degree. Though other studies examined variables such as nose pokes and grooming (e.g., Patterson, 2015), the sole variables of interest in this study were proportion of total time spent with both the familiar and unfamiliar rat and the

proportion of contacts each rat initiated with respect to the rat of interest. The goal was to see what, if any, changes EE produced in these variables as measured by a two-trial social preference task as well as examine what kind of differences EE might produce in the brain.

EE was expected to have a significant effect on both the proportion of total time spent with the novel rat as well as the proportion of contact initiations with the novel rat. Sex was hypothesized to have a significant effect on both measures, as well. With regard to the proportion of total time spent with the novel rat, it was hypothesized that EE rats would spend a lower overall proportion of time with the novel rat compared to unenriched control rats. Similarly, it was hypothesized that EE rats would initiate a lower proportion of overall contact with the novel rat compared to the unenriched controls. Females were expected to spend a lower proportion of time with the novel rat than males, as well as initiate a lower proportion of contacts than males. It was hypothesized that less neural activation would be observed in the BLA of EE rats compared to unenriched controls. Higher neural activation in the CA2 region of the hippocampus was expected in EE rats compared to control rats.

Method

Subjects

For the purpose of this study, the Arts and Sciences Animal Facility at Appalachian State University and Harlan Sprague Dawley provided young Long-Evans hooded rats (N=36). This experiment utilized 18 females and 18 males from seven litters, which were separated into same-sex groups post-weaning. The groups were housed in plastic shoebox cages with aspen bedding and free access to food and water for the duration of the experiment. They lived in a vivarium that was temperature and humidity controlled and kept on a 12-hour light/dark cycle.

The 36 rats were separated into an environmental enrichment (EE) group (6 male, 6 female) and a control (no-EE) group (6 male, 6 female). A subgroup within the control group was a stimulus group (3 male, 3 female). The females in this group were part of and housed with the control group, but they acted as stimulus animals in the social preference task, which will be explained later in greater detail. The males in this group were housed in groups separately from the control group. All stimulus animals were sex and age-matched to the EE and no-EE animals. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Appalachian State University (Protocol #15-02 as amended, M.C. Zrull, P.I.)

Environmental Enrichment

Rats in the EE group (*n*=12, 6 male, 6 female) experienced environmental enrichment between PND 23 and 48 (see Figure 1). EE sessions lasted 1.5h/day and occurred five of every seven days. Two separate EE cages were utilized, one for males and one for females. Each cage had the dimensions 46 X 48 X 79 cm (w X d X h) and was made of wood and wire mesh. Platforms within the cage were 14, 25, 43, and 61 cm above the base of the cage, which was covered in a layer of aspen bedding. Toys for each cage were identical but kept separate to avoid the scent of the opposite sex to interfere with play and exploration within the EE cages. The toys and platforms mentioned were one part of the enrichment the cages provided, as they provided differing textures, sizes, and scents to interact with. Toys were changed on a rotating schedule of four different EE setups (see Figure 2). Another aspect of enrichment the cages offered was the opportunity for EE rats to interact with conspecifics. A total of twenty EE sessions occurred. Control and stimulus rats were handled each day EE occurred to control for the loading and unloading of EE rats.

Social Preference Task and Data Analysis

One social preference (SP) task occurred on PND 49; this task had two trials, both of which were conducted in a 63x42x45 cm (w X d X h) wooden box (see Figure 3). During the first trial, two age and sex matched unfamiliar rats (an EE rat and a no-EE rat or two no-EE rats) were placed in the SP structure together for 3 minutes. Total time in seconds spent together and number of initiations of contact by each animal was recorded. For the second trial, the same two rats paired for trial one were placed in the box along with a third stimulus rat. For each experimental rat, total time spent with the stimulus rat and number of initiations of contact with the stimulus rat were recorded. For each rat, a 30-minute delay occurred between Trials 1 and 2. Trials were observed by closed-circuit video, times and initiations were recorded by multiple observers, and trials were videotaped for further analysis.

For the measurement of total time any two rats spent together, only time spent in deliberate social contact was recorded. Initiation of contact included chasing behavior, of which multiple contact initiations would be recorded for continuous chasing. Other social behaviors like pouncing, climbing, pinning, nuzzling, or simply any initiation of physical contact was recorded as contact initiation. ANOVA was used to analyze behavioral data, including the measures observed and the differences between groups.

Histology

After the second trial, all rats (N=36) were subject to 60 to 90 minutes in a quiet and dark environment. Animals were injected with a lethal dose of sodium pentobarbital (100 mg/kg b.w., ip). Rats' corneal and tail reflexes were tested before perfusion began. Phosphate-buffered saline (PBS) was used to intracardially perfuse the animals; 4% paraformaldehyde in 10 mM phosphate buffer (PB) was then used. Each brain was dissected out of the cranium and post-fixed in 10% sucrose in 4% paraformaldehyde in PB for 5 days. After this period, each was then stored in PB.

From one hemisphere per brain, sagittal sections were taken at 50 µm. In order to observe the activation of the c-*fos* gene in neurons, sections were processed with immunohistochemistry (ihc). C-*fos* is an immediate early gene that is quickly activated when a cellular stimulus is presented. Its activation produces the c-FOS protein, which is able to be visualized through ihc. Thus, level of c-FOS expression can be used as a neural activity marker in relevant areas of the brain.

Floating sections were rinsed in PBS (2 x 5 min) and incubated for 15 min in 1% hydrogen peroxide. Sections were then rinsed a second time in PBS (2 x 5 min) and incubated for 60 min in 15% goat serum in 0.2% Triton-X. Rat anti-c-*fos* made in rabbit (Santa Cruz Biotechnology, SC-52) was then used to process sections for 40 h. Sections were again rinsed in PBS (6 x 10 min) and incubated for 60 min in biotinylated goat anti-rabbit secondary antibody (Vector). Sections were rinsed in PBS (3 x 10 min), exposed to a peroxidase-labeled avidin-biotin complex for 1 h (Vector), and rinsed again in PBS (2 x 10 min). Lastly, an enzyme substrate (VIP, Vector) was applied to sections for at least 2 min.

Subsequently, sections were mounted onto gel-coated slides and air-dried. They then were dehydrated in graded ethanols, cleared with toluene, and cover-slipped with Permount (Fisher). In order to visualize cytoarchitecture, alternate sections were processed for Nissl staining with thionin.

Microscopy and Data Analysis

A Nikon Eclipse microscope and PixeLink digital camera were used to analyze the stereology of brain sections. In order to identify the basolateral amygdala (BLA) and CA2 region of the hippocampus, an atlas of the rat brain (Pellegrino, Pellegrino, & Cushman, 1969) was utilized.

Activation of c-*fos* in the previously mentioned two areas of the brain were compared between EE and no-EE rats using the Plan 10 objective and a 1024 x 768 pixel image. A transparent counting template was placed over the images of the brain and neurons were marked as dark or medium darkness, and the total number of FOS-positive cells was recorded. The darkness of the neurons was an indicator of the amount of c-*fos* activation in each cell. Ambiguous or poorly stained images were not analyzed. Three samples of BLA and 2 samples of CA2 were examined from each section.

Results

Social Preference Task

While it was hypothesized that EE would have a significant effect on the proportion of total time spent with the novel rat, no effect of EE alone was observed when considering this measure ($F(1,20) = 0.02$, $p = .8816$). The hypothesis that sex would have a significant effect on the proportion of contact initiations with the novel rat was also not supported—there was no observed effect of sex alone ($F(1,20) = 3.22$, $p = .0881$). The hypothesis that EE rats would spend a lower proportion of total time with the novel rat was not supported, and the hypothesis that females would spend a lower proportion of time with the novel rats than males was not supported for the EE group. The hypothesis that females would spend a lower proportion of time with the novel rat than males was supported for the no-EE group.

A significant interaction of EE and sex on the proportion of total time spent with the novel rat was observed ($F(1,20) = 6.75$, $p = .0172$), with no-EE males spending a larger proportion of time with the novel rat ($M = 0.63$, $SD = 0.17$) than no-EE females ($M = 0.42$, $SD = 0.09$), $t(20) = 2.99$, $p = 0.007$ (see Table 1). EE males and females did not significantly differ in

the proportion of time spent with the novel rat (M = 0.50, SD = 0.08; M = 0.54, SD = 0.13), $t(20) = 0.57$, $p = .576$.

EE was expected to have a significant effect on the proportion of initiated contact with the novel rat, and a significant effect of EE alone was observed ($F(1,20) = 4.96$, $p = .0375$). The hypothesis that sex would have a significant effect on the proportion of initiated contact was not supported either ($F(1,20) = 2.16$, $p = .1575$). The hypothesis that EE rats would initiate a lower proportion of contacts with the novel rat than no-EE rats was not supported, and the hypothesis that females would initiate a lower proportion of contacts than males not supported for the EE group. The hypothesis that females would initiate a lower proportion of contacts than males was supported for the no-EE group.

There was a significant interaction between EE and sex with proportion of initiated contact with the novel rat ($F(1,20) = 9.73$, $p = .0054$). In contrast to the interaction seen with proportion of time spent with the novel rat, the sex difference was seen only with EE rats, and no-EE rats displayed no such significant sex difference. EE males initiated a larger proportion of contacts with the novel rat (M = 0.50, SD = 0.09) than EE females (M = 0.27, SD = 0.10), $t(20) = 3.22$, $p = .004$ (see Table 2). Males and females in the no-EE category initiated a similar proportion of contacts with the novel rat (M = 0.46, SD = 0.12; M = 0.54, SD = 0.17), $t(20) = 1.12$, $p = .276$.

Histology

The hypothesis that EE rats would show a decrease in BLA neural activation compared to no-EE rats, as measured by the number of c-*fos* positive neurons, was supported (see Table 3). EE animals showed 60% less neural activation in the BLA compared to the no-EE group; this

finding was significant ($F(1,22) = 15.89$, $t(22) = 3.66$, $p = .001$) (see Figure 4). The hypothesis that EE animals would display an increase in activity in the CA2 region of the hippocampus compared to the no-EE group was not supported (see Table 3). EE rats displayed 60% less neural activation in this region than no-EE rats; this finding was significant ($F(1,13) = 0.001$, $t(13) = 4.10$, $p = .001$) (see Figure 5).

Discussion

This study examined the effects of EE on the behavior of adolescent rats during a two-trial social preference task on pnd 49. Neural activation in brain regions (i.e., BLA and CA2) thought to contribute to social recognition and anxiety was examined as well. The task and subsequent neural analysis were carried out to gain insight into how EE might influence animals' preference for familiarity or novelty and two brain regions related to emotion and social preference.

Regarding the proportion of time spent with the novel rat, a significant interaction was observed between EE and sex. EE rats, whether female or male, spent a large proportion of their time with the novel rat; this is consistent with previous findings that EE males and females both spent a high amount of time with an unfamiliar rat (Siviy & Panksepp, 2011). However, while both male and female unenriched rats spent a considerable proportion of their time with the novel rat, males spent a significantly larger proportion of their time with the novel rat than females. The opposite effect is seen when examining the proportion of initiated contacts with the novel rat, though the significant interaction of EE and sex remains. For this measure, unenriched rats, regardless of if they were male or female, displayed a high proportion of contact initiations with the novel rat. Sex differences were only observed when considering EE rats, where males initiated a high proportion of contact with the novel rat, while females initiated a low proportion

of contact with the novel rat, which is consistent with findings from Siviy & Panksepp (2011) that no-EE females initiated more play with an unfamiliar rat. Findings from Siviy & Panksepp (2011) examined social play, which consists of only two rats together in an environment, unlike the three rats utilized in Trial 2 in this study, which may contribute to differences between their findings and these.

These behavioral findings are somewhat inconsistent with literature suggesting that enriched rats tend to display a quicker adaptation to novelty than control rats (Simpson & Kelly, 2011); this indicates that enriched rats should spend a small amount of time with novel rat and initiate less contact with the novel rat, as well as spend less time and initiate less contact with the novel rat than the unenriched rats. EE rats engaging in less contact with a novel rat would be expected due to the enriched rats adapting to the novel rat more quickly than the unenriched group—in other words, the novel rat should lose its novelty faster for the enriched rats. This expectation only held true when considering female enriched rats and the proportion of initiated contact with the novel rat; this group displayed a significantly lower proportion of contact initiations with the novel rat than both male enriched rats and the entire unenriched group.

This study's results imply that EE rats, no matter if male or female, spent a lot of their time during the task with the novel rat. Females, however, tended to stay with the novel rat, while males tended to run away from the novel rat and return to initiate contact with the novel rat. In the opposite way, unenriched control rats, regardless of sex, tended to approach, initiate contact, and then leave the novel rat; they engaged in this behavior quite often during the task. Unenriched males spent the time in between contact initiations with the novel rat, while unenriched females spent that time away from the novel rat, resulting in the significant difference in proportion of time spent with the novel rat seen for no-EE animals.

Behavioral findings suggest that EE has a mediating effect on risky behaviors in adolescence, specifically in terms of social preference behaviors, but only to an extent. This is reflected in the lack of sex differences in the EE group when considering the proportion of total time spent with the novel rat; sex differences remained with the unenriched group for this measure. Simple maturation differences between adolescent males and females is thought to account for the sex differences present when considering the proportion of contact initiations for the EE group. Even though enrichment seems to balance the sexes when it comes to the time rats are spending with an unfamiliar rat, maturity wins out when it comes to initiating contact—females did not initiate a lot of contact, as they were more mature than males, and males initiated a level of contact similar to the unenriched rats.

BLA histology and microscopy were consistent with the literature, which suggests that enrichment should diminish negative emotional responses, such as anxiety, in rats that experience it; these responses are able to be visualized through activation in the BLA (Ashokan et al., 2016, Koe et al., 2016). Enriched rats in the current study displayed 60% less neural activation in the BLA than unenriched rats, which supports the idea that enrichment should reduce an anxiety response, thus result in a lower level of activation in areas related to stress responses, in enriched animals.

Enrichment was thought to result in an increase in activity in the CA2 region of the hippocampus, as the literature has suggested that EE increases the number of cells and the amount of neurogenesis occurring in the region of the hippocampus containing the CA2. This would suggest that enrichment increases an animal's capacity for social recognition and social memory, as the CA2 region is crucial for this process (Tanti et al., 2012). In this study, enriched rats displayed 60% less activity in this region than unenriched rats. Perhaps since enrichment

facilitates the formation of connections, dendritic branching, and an increased number of cells and cell size within the whole network (Smith et al., 2016), activation within this network would be more spread out across the network in enriched animals, resulting in less activation in the CA2, a region central to these ideas (Hitti & Siegelbaum, 2014). With this way of thinking, enrichment works to facilitate engagement in social recognition in animals, which would justify the lower level of activation in this region.

Perhaps in future studies, other, more specific measures of social exploration, such as nose pokes and pounces, could be examined to further whittle down the differences in behavior between enriched and unenriched rats, as well as males and females, when given the choice to interact with a familiar or unfamiliar rat. Further examining the CA2 of enriched and unenriched animals would be helpful, as well, as this area was not able to be visualized in many sections due to tearing and poor mounting. It may be of use to examine other areas of the hippocampus as well to further understand the formation and usage of recognition and memory during social preference tasks, and carrying out this social preference task in younger adolescents would help elucidate where the differences seen in this study arise from. The antibody used in this study was bad, which was a major limitation to the quality of brain tissue produced.

For the current study, it appears that EE has an effect on social preference behaviors in older adolescent rats, though perhaps not quite in the expected way. For some measures, namely the proportion of contact initiations for this study, EE is not enough to overcome the maturity differences between males and females (Willing, Drzewiecki, Cuenod, Cortes, & Juraska, 2016), though it does result in a significantly lower proportion of initiated contact with the novel rat for the enriched females compared to the entirety of the unenriched group. Enrichment mediated

rats' reaction to novelty in the form of an unfamiliar rat across sexes when considering the proportion of total time spent with the novel rat, which was an expected effect of enrichment.

References

Ashokan, A., Hegde, A., & Mitra, R. (2016). Short-term environmental enrichment is sufficient to counter stress-induced anxiety and associated structural and molecular plasticity in basolateral amygdala. *Psychoneuroendocrinology*, *69*189-196. doi:10.1016/j.psyneuen.2016.04.009

Blakemore, S., & Choudhury, S. (2006). Development of the adolescent brain: Implications for executive function and social cognition. *Journal of Child Psychology and Psychiatry, 47*(3-4), 296-312.

Burke, A., & Miczek, K. (2015). Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: Role of social experience and adaptive coping behavior. *Psychopharmacology, 232*(16), 3067-3079. doi:10.1007/s00213-015-3947-5

Cobb, D. E. (2015) *Environmental enrichment promotes adaptation to environment rearrangement in younger but not older adolescent rats.* Unpublished honors thesis, Appalachian State University, Boone, NC.

Ferguson, J., Young, L., & Insel, T. (2002). The neuroendocrine basis of social recognition. *Frontiers in Neuroendocrinology*, *23*(2), 200-224.

Forgays, D. G., & Forgays, J. W. (1952). The nature of the effect of free-environmental experience in the rat. *Journal of Comparative and Physiological Psychology*, *45*(4), 322-328. doi:10.1037/h0053731

Hebb, D.O. (1947). The effects of early experience on problem-solving at maturity. *American Psychologist, 2*, 306-307.

Hitti, F., & Siegelbaum, S. (2014). The hippocampal CA2 region is essential for social memory. *Nature*, *508*(7494), 88-105.

Koe, A. S., Ashokan, A., & Mitra, R. (2016). Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. *Translational Psychiatry, 6*e729. doi:10.1038/tp.2015.217

Lynn, D., & Brown, G. (2009). The ontogeny of exploratory behavior in male and female adolescent rats (Rattus norvegicus). *Developmental Psychobiology*, *51*(6), 513-520.

Mandolesi, L., De Bartolo, P., Foti, F., Gelfo, F., Federico, F., Leggio, M., & Petrosini, L. (2008). Environmental enrichment provides a cognitive reserve to be spent in the case of brain lesion. *Journal of Alzheimer's Disease*, *15*(1), 11-28.

Palagi, E., Burghardt, G. M., Smuts, B., Cordoni, G., Dall'Olio, S., Fouts, H. N., & ... Pellis, S. M. (2016). Rough-and-tumble play as a window on animal communication. *Biological Reviews*, *91*(2), 311-327. doi:10.1111/brv.12172

Panksepp, J. (2011). Review: The basic emotional circuits of mammalian brains: Do animals have affective lives?. *Neuroscience and Biobehavioral Reviews*, *35*(Pioneering Research in Affective Neuroscience: Celebrating the Work of Dr. Jaak Panksepp), 1791-1804. doi:10.1016/j.neubiorev.2011.08.003

Patterson, K. L. (2015). *Environmental enrichment affects the behavior of adolescent rats in social preference task*. 2015. Unpublished honors thesis, Appalachian State University, Boone, NC.

Okuda, H., Tatsumi, K., Makinodan, M., Yamauchi, T., Kishimoto, T., & Wanaka, A. (2009). Environmental enrichment stimulates progenitor cell proliferation in the amygdala. *Journal of Neuroscience Research*, *87*(16), 3546-3553. doi:10.1002/jnr.22160

Simon, N. W., Gregory, T. A., Wood, J., & Moghaddam, B. (2013). Differences in response initiation and behavioral flexibility between adolescent and adult rats. *Behavioral Neuroscience, 127*(1), 23-32. doi:10.1037/a0031328

Simpson, J., & Kelly, J. P. (2011). Review: The impact of environmental enrichment in laboratory rats—Behavioural and neurochemical aspects. *Behavioural Brain Research*, *222*246-264. doi:10.1016/j.bbr.2011.04.002

Siviy, S. M., & Panksepp, J. (2011). In search of the neurobiological substrates for social playfulness in mammalian brains. *Neuroscience and Biobehavioral Reviews*, (9), 1821.

Smith, A., Avram, S. W., Cymerblit-Sabba, A., Song, J., & Young, W. (2016). Targeted activation of the hippocampal CA2 area strongly enhances social memory. *Molecular Psychiatry*, (8), 1137. doi:10.1038/mp.2015.189

Stansfield, K. H., & Kirstein, C. L. (2006). Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiology*, *48*(1), 10-15. doi:10.1002/dev.20127

Stein, L. R., O'Dell, K. A., Funatsu, M., Zorumski, C. F., & Izumi, Y. (2016). Short-term environmental enrichment enhances synaptic plasticity in hippocampal slices from aged rats. *Neuroscience*, *329*294-305. doi:10.1016/j.neuroscience.2016.05.020

Sturman, D. A., & Moghaddam, B. (2011). The neurobiology of adolescence: Changes in brain architecture, functional dynamics, and behavioral tendencies. *Neuroscience and Biobehavioral Reviews*, (8), 1704. doi:10.1016/j.neubiorev.2011.04.003

Tanti, A., Rainer, Q., Minier, F., Surget, A., & Belzung, C. (2012). Differential environmental regulation of neurogenesis along the septo-temporal axis of the hippocampus. *Neuropharmacology*, *63*374-384. doi:10.1016/j.neuropharm.2012.04.022

Thor, D. H., Harrison, R. J., Schneider, S. R., & Carr, W. J. (1988). Sex differences in investigatory and grooming behaviors of laboratory rats (Rattus norvegicus) following exposure to novelty. *Journal of Comparative Psychology*, *102*(2), 188-192. doi:10.1037/0735-7036.102.2.188

Trezza, V., Baarendse, P. J., & Vanderschuren, L. J. (2010). Review: The pleasures of play: pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences, 31*463-469. doi:10.1016/j.tips.2010.06.008

Van Kerkhof, L. W., Damsteegt, R., Trezza, V., Voorn, P., & Vanderschuren, L. J. (2013). Social play behavior in adolescent rats is mediated by functional activity in medial prefrontal cortex and striatum. *Neuropsychopharmacology, 38*(10), 1899-1909. doi:10.1038/npp.2013.83

Will, B., Toniolo, G., Kelche, C., Pallage, V., Deluzarche, F., & Misslin, R. (1986). The effects of postoperative physical environment on novelty seeking behaviour and maze learning in rats with hippocampal lesions. *Behavioural Brain Research*, *19*(3), 233-240. doi:10.1016/0166-4328(86)90023-9

Willing, J., Drzewiecki, C. M., Cuenod, B. A., Cortes, L. R., & Juraska, J. M. (2016). A role for puberty in water maze performance in male and female rats. *Behavioral Neuroscience*, *130*(4), 422-427. doi:10.1037/bne0000145

Table 1.

Proportion of Time Spent with Novel Rat during the Social Preference Task on Postnatal Day 49.

Group	Sex	*N*	*M*	*SD*
EE	M	6	0.50	0.08
	F	6	0.54	0.13
no-EE	M	6	0.63*	0.17
	F	6	0.42*	0.09

Note. The significant interaction between EE and sex is indicated (*$p < .0172$). Abbreviations: EE, environmental enrichment; no-EE, no environmental enrichment.

Table 2.

Proportion of Contact Initiated with Novel Rat during the Social Preference Task on Postnatal Day 49.

Group	Sex	*N*	*M*	*SD*
EE	M	6	0.50*	0.09
	F	6	0.27*	0.10
no-EE	M	6	0.46	0.12
	F	6	0.54	0.17

Note. The significant interaction between EE and sex is indicated (*$p < .0054$). Abbreviations: EE, environmental enrichment; no-EE, no environmental enrichment.

Table 3.

Mean Number of Activated Neurons in BLA and CA2.

	BLA			CA2		
Group	*N*	*M*	*SD*	*N*	*M*	*SD*
EE	12	14.57*	6.66	12	22.00	10.08
no-EE	12	29.56*	19.56	12	39.26	13.33

Note. The significant difference is indicated (*$p < .0007$). Abbreviations: Abbreviations: BLA, basolateral amygdala; CA2, *cornu ammonis* 2 region of the hippocampus; EE, environmental enrichment; no-EE, no environmental enrichment.

Figure 1. Rats were weaned on pnd 22, and twenty EE sessions took place from pnd 23 to 48. These were followed by the SP task and perfusion on pnd 49.

Figure 2. This is an example of one of the enrichment cages with one of four arrangements of objects. Enrichment occurred through the interaction with same-sex familiar and unfamiliar conspecifics, ramps and platforms, as well as a variation of objects.

Figure 3. This picture shows the walled 63x42x45 cm (w X d X h) open field in which the social preference task on postnatal day 49 occurred.

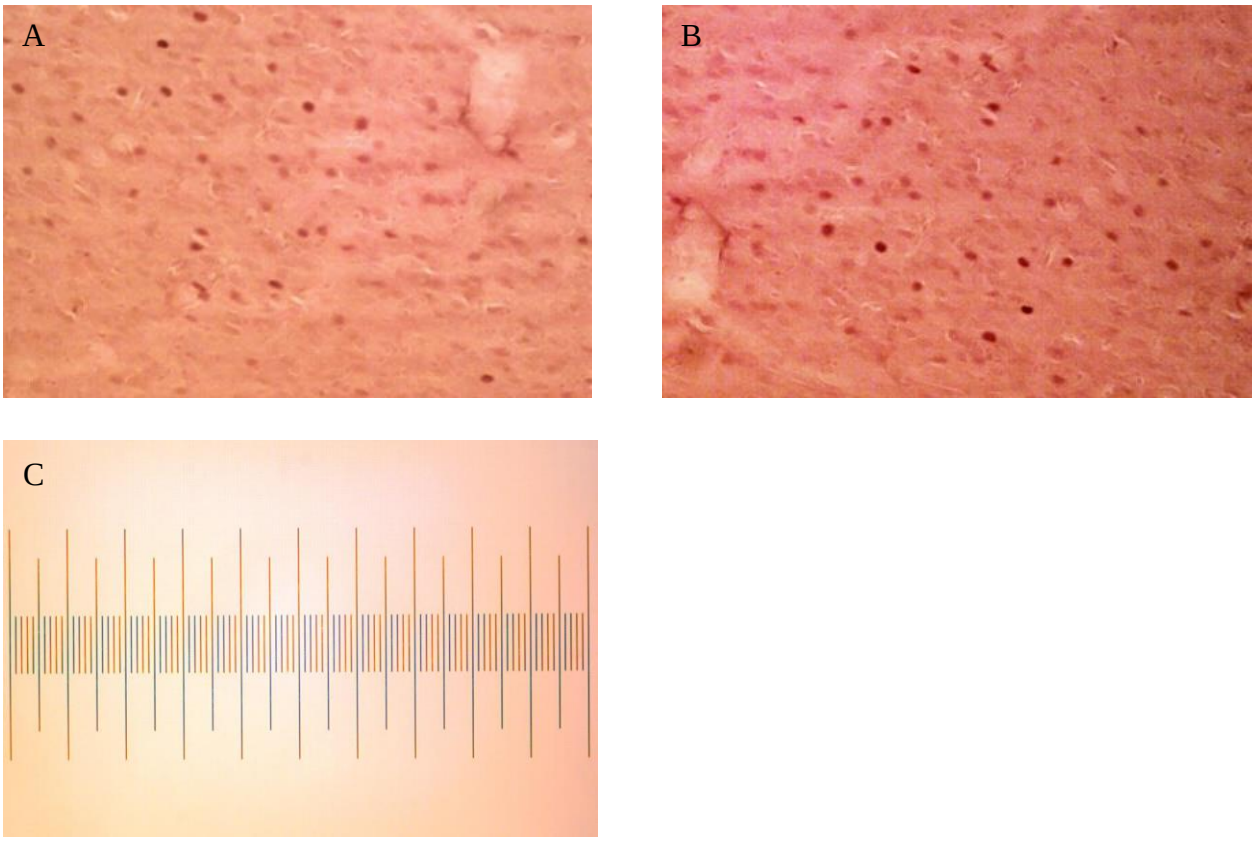

Figure 4. (A) BLA region in EE animals. (B) BLA region in no-EE animals. EE animals showed a 60% reduction in c-*fos* activated cells in BLA compared to no-EE animals. (C) Scale bar indicating section size. Each small division is 10 μm, each large division is 100 μm, and the entire bar is 1,000 μm.

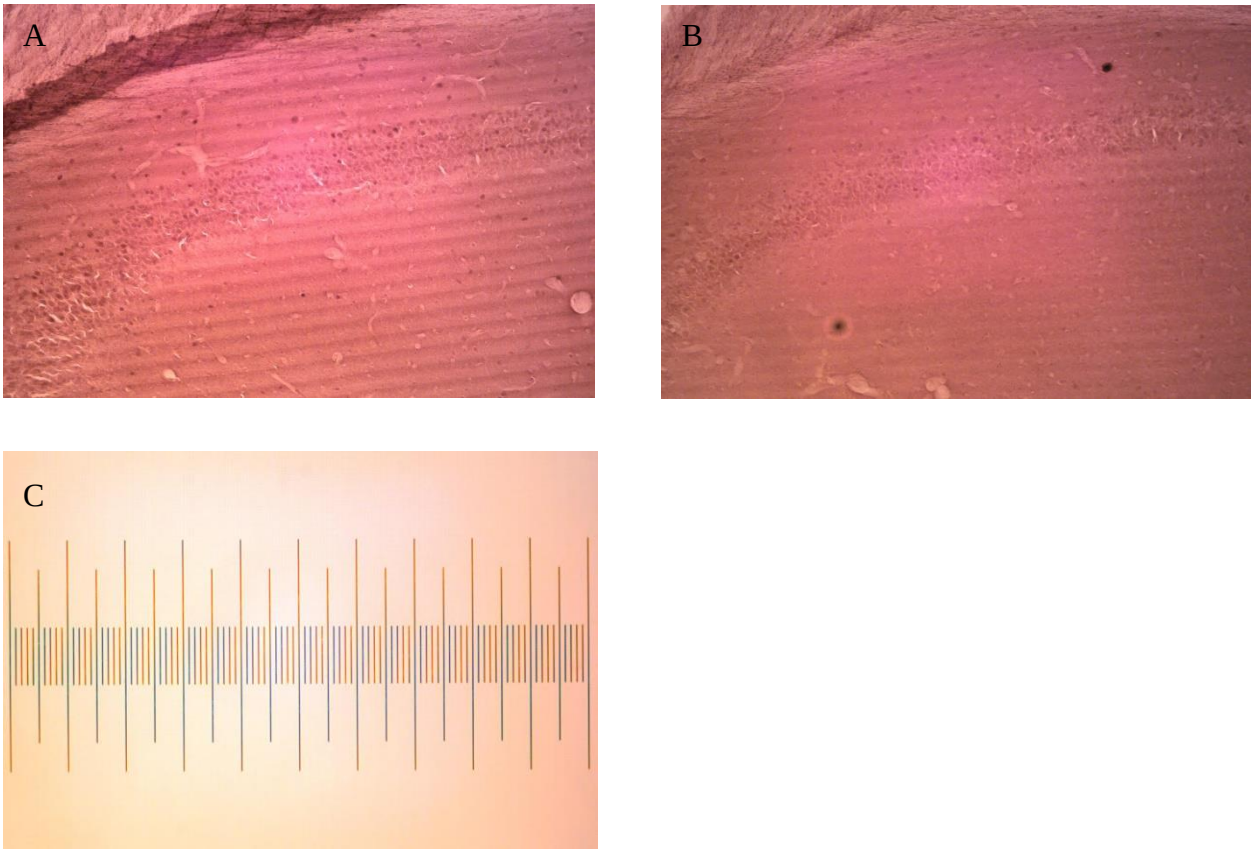

Figure 5. (A) CA2 region of the hippocampus in EE animals. (B) CA2 region of the hippocampus in no-EE animals. EE animals showed a 60% reduction in c-*fos* activated cells in the CA2 region compared to no-EE animals. (C) Scale bar indicating section size. Each small division is 10 μm, each large division is 100 μm, and the entire bar is 1,000 μm.