ENVIORNMENTAL ENRICHMENT AFFECTS THE BEHAVIOR OF ADOLESCENT RATS IN A SOCIAL PREFERENCE TASK

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Honors Thesis

Appalachian State University

Submitted to the Department of Psychology
and the Honors College
in partial fulfillment of the requirements for the degree of

Bachelor of Science

August, 2015

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Abstract

While the behavioral development of male and female rats differs, adolescents of both sexes have an interest in novelty. Environmental enrichment (EE) can add to that interest by providing opportunity for interaction with objects and other rats, and a history of EE does affect the behavior of adolescent rats in object and environment novelty preference tasks. Of interest in this study was determining if interest in social novelty might be enhanced by EE and influence adolescent rats' interaction with a known and novel conspecific in a social preference task. On postnatal day (PND) 21, a group of rats began EE sessions with novel social partners and objects 5 days a week for 1.5 h, which continued until PND 49. On PND 35 and 49, the rats were introduced to two conspecifics, j1 and j2, for a 3-min trial, then were placed back in their home cage. After a 30-min delay, the test animals were replaced in the apparatus for a choice trial, where they were expected to show higher investigation behaviors toward a third animal, j3, rather than one of the original animals, j1. There were no significant differences between EE and no-EE animals in time spent near or nose pokes directed toward the novel animal (j3) during the test phase nor between behavior of males and females, independent of EE status. On PND 35, no-EE females investigated the third rat, j3, significantly less than the other animals. There were no differences in behavior due to main effects of EE or sex for the general investigation variables rearing or self-grooming; however, EE animals groomed more in response to novelty on PND 49 than no-EE animals, and males reared more on PND 35 and females more on PND 49. While many hypotheses were not supported, EE was an important factor and interacted with other variables to alter experimental rats' behavior in the social preference task.

Environmental Enrichment Affects the Behavior of Adolescent Rats in a Social Preference Task

While there is no single event that specifies the beginning or end of adolescence in most species, puberty is often an important signal that it has begun, and adolescence usually is considered over when young adults move out on their own. In humans, adolescence is a period of time that can last many years. Adolescence is a brief period during the life of a rat, usually lasting only about 40 days post-weaning, which usually occurs around postnatal day 21 (Lynn & Brown, 2009). The physical and behavioral development of an adolescent rat can be monitored and controlled much more closely than that of a human, making them an ideal model for the study of changes in social behavior dependent upon events that might occur during this period of development (Simpson, Gregory, Wood, & Moghaddam, 2013). While the unique social units of wild rats would make them particularly ideal for social experimental tasks, as these animals normally live in large colonies of many family members, outbred strains of laboratory rats, which are analogs of their wild relatives, will engage in similar behaviors and build a colony lifestyle if given the opportunity (Barnett & Spencer, 1950). However, there are also differences between wild and outbred laboratory rats that make the outbred animals useful in studies of social behavior during adolescence. In a wild rat colony, intruding outsiders would be pursued and attacked or driven out by adults *and* adolescents of the colony; however, outbred adolescent laboratory rats will engage in play and other social behaviors with novel conspecifics (Barnett & Spencer, 1950; Lynn & Brown, 2009). This tendency in young laboratory rats to engage other rats creates a unique opportunity to test preference for novel or familiar conspecifics in social challenge task.

Adolescent rats, like adolescents of other species, have a high interest in novelty and low impulse control (Simpson et al., 2013). Of interest in the current study was determining if these characteristics would influence an adolescent rat's social interaction choices between a known and novel conspecific in a social preference task. The paradigm added the opportunity of choice for the subject rat to the procedure described by Thor, Harrison, and Schneider (1988). When given a choice between two young animals, one known and one unknown, adult rats show a preference for investigating the unknown animal (Thor et al., 1988). In Thor and colleagues' version of the task, the adult test subject has had exposure to the known young rat over a long period of time. Changing the task to include relatively brief exposure to three animals, two introduced at the onset, and one switched for one of the original rats during a second trial, could potentially change the outcomes. The change in procedure provides the subject rat with the opportunity to examine two novel social partners, and then choose between a third novel social partner and one of the previously introduced animals. Typically social preference testing is done using adult rats as the test subjects and young animals as stimuli (e.g., Engleman, Wotjak, & Landgraf, 1995; Simpson et al., 2013; Thor et al., 1988), however this study used adolescent animals for both the subject and test animals.

Exposure to and experience with novel environments is known to alter the behavior of adolescent rats (Lynn & Brown, 2009). In adolescent rats, a history of environmental enrichment, periods of time in a setting with multiple levels, toys, and conspecifics as well as time for interaction with these things can reduce the effects of novelty on adolescent rats in a test situation (Simpson & Kelly, 2011). Given that enrichment may alter novel object preference in adolescent rats, the introduction of environmental enrichment may also

influence behavior and novelty preference of adolescent subjects in a social preference paradigm. This thesis study was designed to examine how environmental enrichment might impact the behavior and choice of adolescent rats in a social preference task.

Typically, adolescence is when young animals begin to leave their parents and venture out on their own for short periods of time, eventually leaving home and beginning their own reproductive cycles (Lynn & Brown, 2009). In rats, adolescence is usually described as between postnatal day (PND) 21 and 60 with sexual maturity and independence being a result (Lynn & Brown, 2009). Physical changes in adolescent rats follow a clear progression, with the development of males and females varying from one another but both being sexually mature and independent by PND 60 (Lynn & Brown, 2009). Often as a result of physical changes, behavior changes drastically during adolescence (Simpson et al., 2013).

The adolescent period in rats is characterized by a variety of behavioral changes including increased sensation and novelty seeking (Simpson & Kelly, 2011; Stansfield & Kirsten, 2005), which is of particular importance to the current study. Exploratory behavior, including investigation of novel objects, markedly increases across the adolescent period driven by changes in hormone and brain function (Lynn & Brown, 2009; Stansfield & Kirsten, 2005). During adolescence, male and female rats experience a rise in gonadal hormones, which is indicative of puberty, and development of characteristics leading to sexual maturity (Vetter-O'Hagan & Spear, 2011). Adolescent females reach typical adult levels of estradiol at PND 48, while adolescent males show increasing levels of testosterone from PND 48 to 78 (i.e., adulthood) (Vetter-O'Hagan & Spear, 2011), which provides an example of the difference in sexual development of males and females during adolescence. Both male and female rats display adolescent behavior beyond when gonadal hormones reach

adult levels (Vetter-O'Hagan & Spear, 2011). Further, during adolescence the brain is developing rapidly and undergoing extensive remodeling (Sturman, Mandell, & Moghaddam, 2010). There is increased myelination, receptor pruning, axonal growth, large changes in white-matter density and grey-matter volume as the frontal cortex develops, and there is significant development in the mesocorticolimbic dopamingergic circuitry, which is considered relevant to motivated behavior in general (Sturman et al., 2010) and plays important roles in novelty and reward seeking as well as risk-taking behavior (Struman & Moghaddam, 2011).

Adolescent rats also show marked changes in social behavior, increased impulsivity, and increased response to novelty, with a strong correlation between impulsivity and response to novelty (Auger & Olsen, 2009; Stansfield & Kirstein, 2005). This impulsivity impacts adolescent rat behavior during the Cued Response Inhibition Task (CRIT) (Simpson et al., 2013). In CRIT, animals are trained to press a reward lever during a positive "go" cue, such as a light, and ignore the lever during a negative "no-go" cue, such as a tone. Adolescent rats had difficulty initiating responses after the "no-go" cue and difficulty learning to inhibit their impulses to press the lever during the "no-go" cue (Simpson et al., 2013). This may be due to the on-going brain development, including areas such as the prefrontal cortex, because when the same rats were tested again when older they improved greatly on response initiation and inhibition of responses to a "no-go" cue (Simpson et al., 2013). The inability of the young animals to inhibit their impulses is very characteristic of adolescents, and the improvement of response inhibition with age shows the reduction in impulsivity over time (Simpson et al., 2013). The animals in the current study were tested at

two ages, PND 35 and PND 49, and while both ages fell within the adolescent period, they allowed examination of a reduction, or not, in impulsivity over this two-week period.

In a social choice paradigm such as the already described modification of Thor and colleagues' (1988) social preference task, the higher level of impulsivity, sensation seeking, and novelty preference seen in adolescent rats are interesting potential influences on preference to investigate a known or novel conspecific (Stansfield & Kirstein, 2005; Thor et al., 1988). Adolescents should be more likely to impulsively seek out interaction with a novel conspecific over a known rat than would an adult rat with reduced levels of sensation seeking (Simpson et al., 2013; Thor et al., 1988). Adult animals learn to inhibit their impulsivity and sensation seeking as they develop from adolescence to adulthood, which helps them to survive in a wild environment as the animal taking a higher number of risks is more likely to be killed (Lynn & Brown, 2009). Interestingly, environmental enrichment, which is often characterized as the opportunity to interact freely with various objects and conspecifics, can reduce or alter sensation- or novelty-seeking behavior in adolescent rats, at least with regard to inanimate objects in known and unfamiliar settings (e.g., Cobb & Zrull, 2014; Simpson & Kelly, 2011; Stansfield & Kirsten, 2005; Will et al., 1986).

Environmental Enrichment

Environmental enrichment (EE), as an experimental tool with rats as subjects, is a period of time outside the home cage spent in a novel environment with multiple levels, various enriching toys, and most often same-sex conspecifics (e.g., Waddell, 1999). This time allows animals to investigate and engage with objects not normally found in the home environment, often in social groups. EE has been utilized as an experimental tool at least since 1947, when D. O. Hebb noted that rats raised in an enriched environment (i.e., playing

at home with his children) performed better on tasks than unenriched animals when returned to the research laboratory (Hebb, 1947). Results of various studies demonstrate that EE alters behavior by influencing learning (e.g., Forgays & Forgays, 1951; Simpson & Kelly, 2011; Will et al., 1986) and altering neural plasticity in the brain of adolescent rats (Simpson & Kelly, 2011; Will et al., 1986).

Social interaction within EE cages is significantly important for EE to have an impact on behavior (Forgays & Forgays, 1951; Waddell, 1999), and the presence of novel conspecifics during EE is also significant to the development of the individuals being enriched (Simpson & Kelly, 2011). Without interaction with novel social conspecifics, the effects of pure EE are lowered significantly (Forgays & Forgays, 1951). While social housing does contribute to later social behavior, EE that includes social enrichment with novel conspecifics increases socialization time and improves performance on social tasks (Simpson & Kelly, 2011). In the present study, the possibility that EE might reduce sensation seeking and increase learning and memory, thus possibly altering social investigation, was examined using a social preference task modified from that described by Engleman and associates (1995).

Social Preference Task

The classic social preference procedure involves exposing adult rats to a single juvenile conspecific (j1) for a period of 3 min, or until 30 s of investigation time has been reached (Engleman et al., 1995). Then, the adult rat is moved into a new cage and after a varying interval of time passes, usually between 30 min to 120 min, the adult is re-exposed to j1 and to a second, unknown juvenile conspecific (j2). The percentage of time spent investigating both juveniles is calculated, and if the adult spends more than chance, or 50%,

of its time with j2, it is understood to have recognized j1 (Engleman et al., 1995). Typically, adult rats are subjects in social preference experiments and juveniles have been the stimulus animals (e.g., Engleman et al., 1995; Thor et al., 1988; Thor, Wainwright, & Holloway, 1982); however, in the current experiment, adolescent rats were used as both test subjects and stimulus conspecifics. In addition to a measure of preference or recognition, behavior in social preference tasks is often measured to determine the general levels of investigation and attentiveness of the rat being tested (Thor et al., 1988).

Increased self-grooming behavior is unique to novel social paradigms, as adult rats engaged in significantly less self-grooming when exposed to a novel object than to a single, novel conspecific (Thor et al., 1988), and this effect was expected in the current study with exposure of the subject rat to a pair of same-aged conspecifics. Self-grooming is a clearly documented indication of self-comfort, which is often performed in comfortable settings as well as in response to novelty (Thor et al., 1988). In fact, novel settings may evoke a stress response in adult rats, and an increase in grooming during novel social situations has been described as a means of self-comfort in an attempt to seek security (Thor et al., 1988). Adult rats of both sexes will spend up to 40% of their waking time self-grooming; however, novelty-induced self-grooming has been found to be significantly more prevalent in male rats than female rats (Thor et al., 1998).

Rearing is a behavioral indication of investigation and curiosity as opposed to crouching and freezing, which is a fear response usually evoked by the scent of a predator in the laboratory (Takahashi, Hubbard, Lee, Dar, & Sipes, 2007). Exposure to a cloth scented with cat odor evoked longer periods of crouching and freezing in rats and a reduction in rearing and investigatory behavior, which led Takahashi and colleagues (2007) to suggest

rearing is indicative of investigation behavior and not fear. Thus, rearing may be an indication of the level of investigation a rat is engaging in while exploring a novel environment. In this study, rearing was considered an indicator of environmental investigation and was useful in determining the general level of investigation shown by a subject rat in the social preference paradigm when exposed to known and unfamiliar stimulus rats.

Sex Differences

Because they differ in social discrimination abilities, both male and female adolescent rats were tested in the current paradigm. Veenema, Bredewold, and DeVries (2012) showed that adult males exhibit show high levels of social discrimination ability and investigate a novel juvenile far more than a known juvenile. In contrast, adult female rats show significantly lower levels of investigation than males for juvenile conspecifics; however, their memory for these conspecifics seems to be intact as both sexes discriminate between a novel and a known juvenile similarly (Veenema et al., 2012). Further, the EE manipulation in the present study was expected to influence male and female rats differently. Enrichment sessions may have a greater impact on males than females by allowing young EE males to practice spatial orientation and social interaction, which would give them an advantage later in life when fighting for dominance or being sexually active (Pellis & Pellis, 2007), and altering behavior in a social preference challenge. Adult males in social species, such as rats, must fight for dominance to be sexually active, so younger males participate in play fighting activities to learn how to appropriately wrestle with other males (Auger & Olsen 2009). Thus EE males may pursue social interaction differently than EE females leading to far more play

fighting, which may enhance social investigation in the male adolescent rats of the current study (Auger & Olesen, 2009; Pellis & Pellis, 2007).

In the present study, only preference for same-sex known or novel conspecifics was examined to avoid confounding investigation with sexual behavior. While it makes sense that the presence of a female can alter behavior of a male rat, exposure to a male of any age can evoke sexual behavior in a female rat (Markham & Juraska, 2007) and perhaps suppress investigation. Investigative and exploratory behaviors are affected strongly by EE (e.g., Bouchon & Will, 1982; Forgays & Forgays, 1951; Will et al., 1986), which differ in male and female adolescent rats (Lynn & Brown, 2009), and EE could promote sex differences in social preference tasks (e.g., Simpson & Kelly, 2011). For example male and female adult rats spend approximately the same amount of their time awake self-grooming; however, males groom more in response social novelty than females (Thor et al., 1988), which may or may not be moderated by EE particularly in adolescent rats who respond differently to novelty (Stansfield & Kirsten, 2005). Thus, it is expected that environmental investigation indicators such as grooming and rearing in adolescent rats may show sex differences in a social preference paradigm due to potential sex by EE interactions.

Current Study

This study is an investigation of behavior during social preference task and how it is affected by EE. Specifically, social investigation and non-specific investigation behaviors were assessed. The study is unique in using cohorts of adolescent rats and exploring the influences of EE on behavior in the social preference paradigm. Because EE can have an effect on exploratory behavior and novelty preference (e.g., Bouchon & Will, 1982; Cobb & Zrull, 2014; Simpson & Kelly, 2011) and because young and adult, male and female rats

exhibit differences in exploratory, novelty preference, and social behavior (e.g., Auger & Olsen, 2009; Lynn & Brown, 2009; Markham & Juraska, 2007), the following hypotheses were made.

It was hypothesized that EE rats would show different levels of investigation of a novel conspecific relative to a familiar conspecific in comparison to unenriched control rats. It was also hypothesized that for both enriched (i.e., EE) and control (i.e., no-EE) rats, males would investigate a novel conspecific relative to a familiar conspecific differently than females. These two hypotheses were tested by comparing EE and not enriched controls as well as males and females on two dependent variables during the second trial of a two-trial social preference task: the proportion of time spent in contact with a novel versus known stimulus rat; and, the proportion of nose pokes at a novel versus known stimulus rat. With regard to general environmental investigation indicators, it was hypothesized that both EE and control males would show different levels of self-grooming behavior in response to a novel social stimulus than females. Finally, it was hypothesized that rearing events, while not affected by enrichment or sex, would decrease across the 3 min of a test trial with a novel and familiar stimulus rats present.

Method

Subjects and Design

Long-Evans hooded rats (*N*=25), provided by the Arts and Sciences Animal Facility, were subjects in the current experiment. There were 13 males and 12 females that came from four litters, which were mixed together into same sex groups at weaning. After weaning, the animals were group housed in shoebox cages with aspen bedding and free access to food and water. They were kept on a 12 h light/dark cycle in a temperature and humidity controlled

vivarium. All animals were handled regularly to encourage comfort with handling, reducing the effects of handling stress on the social preference procedure.

The 25 rats were divided into an environmental enrichment (EE) group (7 male, 5 female) and an unenriched control (no-EE) group (6 male, 7 female). The EE cohort experienced enrichment sessions, which are described in the following section. An additional cohort of social preference stimulus rats (*N*=12) was kept separate from both the EE and no-EE cohorts, to prevent scent recognition prior to testing. These additional animals were sex and age matched to the experimental cohort, and their purpose was to serve as stimulus animals for the social preference protocol. Like the EE and no-EE rats, the stimulus animals were housed in groups of three or more to ensure social deprivation did not affect the experiment (e.g. Thor et al., 1982), and all rats were cared for and used according to IACUC standards and requirements (Protocol #15-02, approved August 14, 2014).

Environmental Enrichment

There were two enclosures and sets of toys for EE sessions, one designated for males and one for females. Each enclosure was 46 X 48 X 79 cm (w X d X h) wooden frame and wire mesh structure with platforms located at 14, 25, 43 and 61 cm above the floor. The lowest of these platforms was a block of wood, with the upper platforms accessible through wire mesh ramps. Sets of toys for male and female EE cages were identical but separate, preventing the scent of the opposite sex from affecting playtime or investigation in the rats. Thus, each EE cage provided a variety of toys, levels, textures, and scents to investigate, in addition to novel social conspecifics (see Figure 1). The EE rats, 5 female and 7 male, were placed in EE cages beginning on PND 25 for 1.5 h, for 9 days over a 2 week period before the beginning of the first social preference procedure. EE sessions continued after the first

social preference procedure on the same schedule, excluding the day on which the social preference procedure was completed, which resulted in 18 EE session overall. No-EE rats, 7 female and 6 male, were handled each day to mimic how the EE animals were handled to reduce any handling confounds between groups. Cage mates were always returned to the same living space, which was ensured by colored tail markings distinguishing different animals. The first social preference procedure was PND 35, and the final social preference procedure was PND 49. Thus the final timeline progressed with nine EE sessions, a social preference task, nine more EE sessions and the final social preference task.

Social Preference Task

The social preference (SP) procedure used in this experiment was modified from Engleman et al. (1995) by the addition of a third rat during both the original exposure and test trials of the procedure. The SP task was conducted using a 33 X 23 cm (w X d), 29 cm high wooden box, which had three sections (see Figure 2). A 2.5 cm wire mesh wall divided the lower half of the compartments, and the upper halves were separated by wooden walls (see Figure 2). Age and sex matched stimulus rats were placed individually into the two 10 X 23 cm outside compartments and allowed to habituate for a few minutes before any trial began. Each experimental rat was placed individually into the center 12 X 23 cm compartment on all trials. The chamber allowed interaction through the wire mesh, thus scent recognition was plausible (cf. Noack et al., 2010); however, it kept the adolescents separate to reduce any possibility of injury due to fighting within this untested age group.

The SP task was a two trial procedure conducted on PND 35 and again on PND 49. Each experimental adolescent rat was allowed to investigate two age and sex matched stimulus rats, j1 and j2, during the first 3 min trial. The experimental rat was removed after

the trial. A 30 min delay then passed, and subsequently the experimental adolescents were re-exposed to j1 and a novel age and sex matched conspecific, j3. The 30 min delay was used because it was less than delays that resulted in demonstrated inability of male rats to discriminate between novel and known conspecifics (e.g., after 45 min, Noack et al., 2010). Trial 1 was completed for a series of same-sex rats, and Trial 2 began for the group of animals to allow for the 30 min delay between trials. While the apparatus was cleaned between each animal, male cohorts were always tested first as a precaution to prevent distraction caused by female scents in the procedural apparatus. Trials were observed via closed circuit video for analysis and videotaped for further data collection.

Data Collection

Investigation of each stimulus rat, j1 and j2 in Trial 1 and j1 and j3 in Trial 2, by each EE or no-EE rat was recorded at the time the SP task was conducted. For Trial 1 both stimulus animals were novel, while in Trial 2 one animal was novel and one was known from the previous trial. Time spent along the wire mesh wall near a stimulus rat was recorded. Using the videotapes of trials, additional data was collected. A nose poke was counted in this experiment whenever the experimental rat turned toward one stimulus rat and pushed its nose toward the stimulus rat. Nose pokes were considered to indicate paying attention to and/or investigating the stimulus rat. The proportions of investigation time and nose pokes directed at the novel stimulus rat during Trial 2 were used as measures of social investigation. Grooming time was counted holistically, with grooming of the facial, dorsal, ventral, and genital areas adding together as global self-grooming behavior. Grooming time was collected from videotapes for all trials. Rearing events were recorded by counting the number of times a rat reared up during each minute of a given trial. Grooming time and rearing events during

Trial 2, when both novel and familiar stimulus rats were present, were used as measures of general investigation behavior.

Results

Social Investigation Measures

There were two hypotheses about social investigation, and each was tested using two social investigation dependent variables. The two measures of social investigation were the proportion of investigation time and proportion of nose pokes directed at the novel stimulus rat during Trial 2 of the SP task. For the proportion of time directed at the novel stimulus rat, it was hypothesized that the EE animals would show a different level of investigation than the no-EE animals. This hypothesis was not supported ($F(1, 16) = 0.60$, $p < .4507$). Animals in both the EE ($M = 0.53$, $SD = 0.12$) and no-EE ($M = 0.57$, $SD = 0.15$) conditions spent similar time with the novel conspecifics. It was also hypothesized that males would investigate novel conspecifics differently than females across enrichment conditions. This hypothesis was not supported ($F(1, 16) = 0.11$, $p < .7464$), with male ($M = 0.56$, $SD = 0.14$) and female rats ($M = 0.54$, $SD = 0.14$) spending a similar proportion of time near the novel stimulus rat. Table 1 shows the means for proportion of time spent investigating the novel stimulus rat during Trial 2 across enrichment conditions, sex, and both PND tests.

For the proportion of nose pokes directed at the novel stimulus rat during Trial 2 of the SP task, the hypothesis that EE animals would show different levels of investigation relative to no-EE animals was not supported ($F(1, 16) = 0.56$, $p < .4664$). Both the EE ($M = 0.55$, $SD = 0.08$) and no-EE ($M = 0.51$, $SD = 0.12$) rats made a similar proportion of nose pokes toward the novel conspecific. The nose poke data also failed to support the hypothesis that males would investigate a novel conspecific differently than females across enrichment

conditions ($F(1, 16) = 0.92$, $p < .3519$), with male ($M = 0.55$, $SD = 0.07$) and female rats ($M = 0.51$, $SD = 0.13$) making a similar proportion of pokes toward the novel stimulus rat. Table 2 shows the means for proportion of nose pokes toward the novel stimulus rat during Trial 2 across enrichment conditions, sex, and both PND tests. None of the hypotheses about social investigation were supported, as neither investigation time nor nose pokes revealed any significant differences in behavior between EE conditions or sexes.

Interestingly, an interaction of EE condition, sex, and PND of the test on the proportion of nose pokes appeared, $F(1, 26) = 3.92$, $p < .0584$. On PND 35, females in the no-EE condition showed significantly lower levels of investigatory nose pokes compared with EE females, EE Males, and no-EE males (see Table 2). When tested on PND 49, the same no-EE females reached levels of investigation comparable to all other groups, which remained relatively stable.

Non-specific Investigation Measures

It was hypothesized that males, across both EE and no-EE conditions, would show different levels of self-grooming behavior in response to a novel social stimulus than females. This hypothesis was not supported ($F(1, 16) = 0.09$, $p < .7628$) with males ($M =$ 12.8-s, $SD = 12.0$-s) and females ($M = 13.2$-s, $SD = 10.6$-s) showing little difference in grooming time. However, an interesting interaction effect between EE condition and PND on grooming time was found, $F(1, 26) = 4.80$, $p < .0375$. On PND 35, EE ($M = 13.1$-s, $SD = 6.3$-s) and no-EE rats ($M = 11.9$-s, $SD = 4.9$-s) showed little difference in grooming time; however, on PND 49, EE animals ($M = 19.6$-s, $SD = 19.5$-s) groomed more than no-EE rats ($M = 7.8$-s, $SD = 4.8$-s) indicating a lower environmental investigation level for the EE rats when a novel conspecific was present. Grooming times are in Table 3.

As hypothesized, rearing behavior decreased over the duration of Trial 2, when both familiar and novel stimulus rats were present, for all experimental rats indicating decreasing levels of investigation in the environment ($F(2, 32) = 14.18$, $p < .0001$). During the first minute of Trial 2, rats reared an average of 6.0 times ($SD = 2.7$). Rearing decreased during the second ($M = 4.2$, $SD = 2.2$) and third minutes of the trial ($M = 3.9$, $SD = 2.0$).

An interaction effect of EE and PND on rearing events was found ($F(1, 16) = 10.44$, $p <.0052$). EE animals had a lower number of rears on PND 35, and rearing increased at the PND 49 SP task (see Table 4). No-EE rats did the opposite with higher levels of rearing during Trial 2 of the PND 35 SP task and lower levels of rearing on PND 49 (see Table 4). This interaction indicates that EE animals increase investigation between the two SP tests and that no-EE rats decrease investigation between the PND 35 and 49 tests. Another unexpected interaction on rearing was found, which was between sex and PND, $F(1, 16) = 4.67$, $p < .0461$. Females had lower levels of rearing at PND 35 when compared with males; however, on PND 49 levels of rearing for males decreased and female rearing increased compared with PND 35 (see Table 4).

Discussion

The hypotheses of this study focused on the effects of EE on investigation behavior during a social preference task using a cohort of adolescent rats. Specific social and general investigation behaviors were measured to determine the possibility of effects of EE and sex on the investigation behaviors. While most of the hypotheses were not supported by data gathered in this study, interesting interactions between EE, sex and PND of testing, and EE and sex affected social and general investigation behaviors, respectively.

The social investigation hypotheses were that EE animals would show different amounts of investigation time near a novel animal than no-EE rats and that males and females would also show differing levels of social investigation time of a novel animal. Social investigation behavior was not changed by enrichment experience or sex. EE animals performed the social preference task similarly to no-EE animals, with both groups spending approximately the same amount of time investigating novel animals. Previous studies testing young adults after adolescent enrichment found that EE animals spent different amounts of time investigating novelty than unenriched controls, at least with regard to novel environments and objects (e.g., Bouchon & Will, 1982; Cobb & Zrull, 2014). EE also significantly affected investigation and exploration time when novelty was not always a factor and there was no social aspect (e.g., Forgays & Forgays, 1951; Simpson & Kelly 2011). Thus the similarity in social investigation time between EE and no-EE rats of the present study was interesting and not predicted from prior research.

Like EE and no-EE rats, Males and females, when enrichment was not considered, also spent about the same amount of time investigating the novel stimulus animal as both younger (PND 35) and older (PND 49) adolescents. This result contradicted the hypothesis that predicted a difference in social investigation behavior between males and females. Females have been found to investigate social partners significantly less than males in a previous study (Veenema et al., 2012). Males and females also routinely investigate novel social conspecifics differently, which is to be expected given their differing roles in a wild colony setting (Lynn & Brown, 2009) and makes the similarity of investigation times across the sexes and EE conditions in the present study interesting. However, the rats in this study are not truly wild animals living in colony hierarchy, and perhaps the lack of true competition

for survival may have influenced the lack of sex differences in behavior of the laboratory rats seen in this study.

Like social investigation time, the proportions of nose pokes directed at a novel animal did not show any differences between enrichment condition rats or by sex. This particular variable was used to test effort to contact and/or interact with the novel or known animal rather than only the time spent investigating near the stimulus rats. It was hypothesized that both EE and sex would have an effect on the proportion of nose pokes toward a novel rat as the second social investigation variable. However, EE and no-EE animals investigated the novel rat with similar proportions of nose pokes, and male and female rats also investigated novel animals with approximately the proportion of nose pokes. Previous studies have shown differences in investigation of novel objects, as measured by contact, due to EE (e.g., Cobb & Zrull, 2014; Sturman & Moghaddam, 2011) and sex differences in social interaction in social behavior tasks (Veenema et al., 2012), thus the expectation that results from the present social preference task would show the same trend is well founded. However, the experimental animals, the test subjects, in prior studies measuring novelty investigation were adults (e.g., Cobb & Zrull, 2014; Thor et al., 1982; Veenema et al., 2012), and perhaps the differences between the current results and past studies lies with the differing age groups used. Further, differences in social task design between this and previous studies are important. Typically, adult experimental rats are allowed direct contact with a known or unknown juvenile in the subject rat's home cage (e.g., Engleman et al., 1995; Thor et al., 1982; Veenema et al., 2012). Thus, the nature of contact between experimental and stimulus rat and the setting of the experimental apparatus may have an effect on the behavior of the experimental rats. The introduction of two juvenile

animals to discriminate between in the first trial of the task used in this study may also have caused differences in behavior. In previous research, only two juvenile animals were used for discrimination, the original (j1), introduced alone during the first trial of testing, and a second (j2), introduced during a second trial of testing (Thor et al., 1982). The addition of a discrimination choice at each test phase, between j1 and j2 and then j2 and j3, may have affected the scent recognition for this experiment (e.g., Veenema et al., 2012). Additionally, the experimental animal in this study design is usually an adult, not an adolescent (e.g., Engleman et al., 1995; Thor et al., 1982; Veenema et al., 2012). Adolescents do behave differently than adults (Simpson et al., 2013; Stansfield & Kirsten, 2005), and the use of adolescent animals, rather than the traditional adult model, for both experimental and testing animals may have affected the results of this study.

Across both test days (i.e. PND 35 and PND 49), males investigated the novel animal for, statically speaking, the same amount of time. Females that had experienced EE also investigated the novel rat for approximately the same amount of time at both tests and for the same amount of time as the males; however, on PND 35, the no-EE females investigated the novel animal significantly less than the other three groups. Some element of the EE experience promoted investigation of social novelty in the EE females and raised their level of investigation at a younger age. The no-EE females did "catch up" and approach EE female and male investigation time at the PND 49 test, but the difference between PND 35 EE females and PND 35 no-EE females was significant. While not hypothesized, this interaction between EE, sex and PND is very interesting as far as behavior is concerned. Females have been shown to investigate a novel animal less than males in previous studies (Veenema et al., 2012), however these studies have normally focused on adult animals, not adolescents. A

series of EE experiences raised investigation levels of young female rats to those of young males, and continued EE experiences may have changed the investigation patterns of an older female. All females seem to approach the same level of investigation as males by PND 49, which is in contrast to the lower levels found by Veenema et al. (2012). Given that EE during adolescence has been shown to affect the behavior of mature animals (Hebb, 1947; Stansfield & Kristen, 2005) and considering the effect on behavior of young rats in this study, if a female rat had continued experience with EE to the mature age used in Veenema et al.'s 2012 study, it is possible that there would be differences in the behavior of those adult females with investigatory behavior resembling that of males.

As in previous research, self-grooming and rearing behaviors were used to demonstrate general non-specific or environmental investigation despite being primarily self-focused behaviors (Takahashi et al., 2007; Thor et al., 1988). As investigatory behaviors, these activities have been shown to indicate investigation of the general environment and in social situations (Takahashi et al., 2007; Thor et al., 1988), making them good measures of general investigation behavior. It was hypothesized that males and females, regardless of EE condition, would differ on measures of self-grooming time, however, no such effect was found. Previous studies have shown significant differences in grooming behavior between sexes in response to novel situations (Thor et al., 1988; Stansfield & Kirsten, 2005), so the lack of difference in this study differs from other findings. While there was no effect of sex on this measure, EE and PND interacted to have a significant effect on the grooming times exhibited by animals when exposed to one known and one novel stimulus rat. On PND 35, EE and no-EE animals displayed similar grooming times, however that changed on PND 49. During the PND 49 test, EE animals displayed over twice the grooming time of no-EE

animals indicating that something about the experience of EE and the age of an animal interacted to affect the general investigation behavior of these rats. EE animals increased grooming time by an average of 6.5-s from PND 35 to PND 49, while no-EE animals decreased by 4.1-s, creating a difference in grooming time of approximately 10-s on PND 49 between EE and no-EE animals. Perhaps the EE animals were more familiar with novelty and demonstrated more general investigation behaviors, which would indicates that EE does change more than social investigation in response to social novelty.

The interaction of EE and testing PND on grooming time raises the question: what about the EE experience might increase grooming time in EE animals between PND 35 and PND 49 and counteract an apparent decrease in grooming time shown by unenriched controls by PND 49? While previous studies have not found this significant a difference in grooming time between EE and no-EE animals in novel social situations, this may be due to the differences in apparatus used (e.g., Thor et al., 1988). While Thor et al. (1988) conducted their experiments in the home cage of experimental animals, this study conducted the experimental testing in a novel apparatus, used only for experimental data collection. Furthermore, the additional sessions of EE between PND 35 and PND 49 affected the behavior of these EE animals significantly, as it has been found to do in previous studies to other general investigatory behaviors (e.g. Hebb, 1947; Stansfield & Kristen, 2005) and may have the capacity to change grooming behavior as well.

The final hypothesis of this study was that rearing, the second indicator of general or environmental investigation used in this study, would decrease across the trial when exposed to both a known and novel stimulus rat.. As the animals grew more comfortable with their environment, they would rear in investigation proportionally less each minute. This

hypothesis was supported by the data, with animals rearing in investigation -30% from Minute 1 to Minute 2, and -7% from Minute 2 to Minute 3 across test trials on PND 35 and 49. Previous research has found that investigation rears decreased over the duration of a period of investigation (Takahashi et al., 2007), and the findings from the present study further support this result. Beyond the hypothesis of this study, and without regard to time within test trials, on PND 35, EE animals reared less than no-EE animals, indicating less investigation of the environment. This result seems reasonable as EE animals experience more social novelty within the EE cage on a regular basis than their no-EE conspecifics (Auger & Olsen, 2009; Stansfield & Kristen, 2005) and become comfortable with the testing environment quickly, thus the differences in rearing on PND 35 may be attributed to the previous experiences of these animals. However, on PND 49, the EE animals increased significantly in total number of rears, while no-EE animals decreased marginally. EE animals are traditionally expected to decrease their response to novelty as they age, at least with respect to novel objects or environments (Cobb & Zrull, 2014; Sturman & Moghaddam, 2011), so this change in behavior was unexpected. If there were any expected interaction, based on previous research (Auger & Olsen, 2009; Hebb, 1947; Lynn & Brown, 2009), it would have been that EE animals would have started lower, and stayed lower, than no-EE animals in rearing behavior. It seems that something about the continued experience of EE raises the investigatory behavior in novel social situations over time during the adolescent period.

Finally, sex and PND interacted, without regard to time, in an unanticipated way to affect rearing behavior. Females started out less curious about their environment and reared less on the PND 35 test and more on PND 49, while males were originally more curious

about their environment and reared more on PND 35 than PND 49. Male and female social development does show significant differences over time during the adolescent period (Auger & Olsen, 2009; Barnett & Spencer, 1950), which may be what is reflected in the rearing data of this study. Socialization behavior often changes rapidly in adolescence, (Auger & Olsen, 2009; Barnett & Spencer, 1950; Pellis & Pellis, 2007), which may be demonstrated by the interaction between sex and testing PND on rearing behavior.

Limitations

While this experiment provided a satisfactory initial test of the social investigatory behavior of enriched and unenriched adolescent rats, it may have been beneficial to begin with a testing set up like those used with adults in the past. Perhaps using the typical testing apparatus, the home cage (e.g., Engleman et al., 1995; Thor et al., 1982), would have allowed a better comparison of the behavior of the adolescents in the study to results of previous research. Similarly, testing the "new" apparatus and procedure of this study with adult animals and comparing the resulting social and behavioral data to previous studies (Engleman et al., 1995; Thor et al., 1982; Veenema et al., 2012) might have provided significantly more insight into the effects of the apparatus on the experiment. Further, the choice to use multiple stimulus animals on each trial of the social task (j1 and j2 on the first trial, and j2 and j3 second trial) certainly affected the social behavior of these rats in an in some way, and testing the procedure with a more familiar group of animals, adult rats, may have provided background for the behavioral differences exhibited by these adolescents. In previous studies, the direct contact with the known and unknown juveniles (e.g., Engleman et al., 1995; Thor et al., 1982; Veenema et al., 2012) has been present throughout, and the unknown factor of the difference in available contact in this experiment could have been

reduced by adult pre-testing as well. Finally, the adults in previous experiments (e.g., Engleman et al., 1995; Thor et al., 1982) have been tested in larger fields than were provided in this study. The difference in size of the field may have further affected the social interactions for these adolescent rats, and increasing the size of the field available to the experimental animals (the middle chamber of the apparatus), may provide clearer data on the social preferences these animals are exhibiting. Additionally, circulating cortisol levels do indicate stress levels (Vetter-O'Hagan & Spear, 2011), thus it may have been helpful to measure circulating cortisol levels immediately after testing on PND 35 and PND 49 to assess relative stress levels of the rats caused by each test as affected by EE.

Conclusions

This study was conducted to investigate the behavior of adolescent rats during social preference task and how it might be affected by EE. Social investigation and non-specific, or general environmental, investigation behaviors were assessed. It was found that neither EE nor sex had main effects on social investigation variables including the proportion of time near or nose pokes toward a novel stimulus rat. Similarly, investigatory rearing and grooming behavior were not affected by EE or sex alone; however, EE did interact with multiple factors to alter the behavior of the experimental animals in this study. The interaction between EE, sex, and test PND on the proportion of social investigation time directed at the novel rat indicated that these factors might have combined to affect the social development and behavior of adolescent rats. The sex of animals and age at testing contributed to how they behaved when faced with a known and a novel conspecific, reflecting the influence of these variables on development of adolescent social preference. Finally, EE was an important factor in multiple ways by interacting with other variables to cause changes in non-specific

investigatory behavior. Little research has been done on the effects of EE on a social preference in adolescent animals, with this study being an initial attempt, and it would be worthwhile to pursue gathering further data, as the results of this study raise interesting questions about the effects of enrichment on social behavior as depending also on age or sex.

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Table 1

Mean (Standard Deviation) Proportion of Investigation Time at the Wall next to the Novel Stimulus Rat on Trial 2 of the Social Preference Task

Enrichment Condition	Female *N*	Female *M (SD)*	Male *N*	Male *M (SD)*
		Postnatal Day 35		
Enriched (EE)	5	0.47 (0.15)	7	0.54 (0.13)
Control (no-EE)	7	0.53 (0.15)	6	0.64 (0.18)
		Postnatal Day 49		
Enriched (EE)	5	0.60 (0.08)	7	0.53 (0.13)
Control (no-EE)	7	0.55 (0.14)	6	0.55 (0.13)

Note. Social investigation time was timed during trials. An experimental animal was considered to be investigating a stimulus animal when it was faced toward or slightly sideways and toward the stimulus animal and not actively engaging in other behaviors, such as grooming.

Table 2

Mean (Standard Deviation) Proportion of Nose Pokes at the Novel Stimulus Rat on Trial 2 of the Social Preference Task

Enrichment Condition	Female *N*	Female *M (SD)*	Male *N*	Male *M (SD)*
		Postnatal Day 35		
Enriched (EE)	5	0.55 (0.08)	7	0.56 (0.08)
Control (no-EE)	7	0.39 (0.15)	6	0.57 (0.04)
		Postnatal Day 49		
Enriched (EE)	5	0.55 (0.07)	7	0.55 (0.10)
Control (no-EE)	7	0.56 (0.11)	6	0.54 (0.03)

Note. A nose poke was counted whenever the experimental animal turned toward a stimulus animal and pushed its nose forward. Nose pokes were tallied holistically, then the proportion of pokes toward either stimulus rat was calculated and analyzed.

Table 3

Mean (Standard Deviation) of Global Grooming Time for Trial 2

Enrichment Condition	Female *N*	Female *M (SD)*	Male *N*	Male *M (SD)*
		Postnatal Day 35		
Enriched (EE)	5	12.60 (4.83)	7	13.43 (7.48)
Control (no-EE)	7	12.14 (5.24)	6	11.67 (6.56)
		Postnatal Day 49		
Enriched (EE)	5	19.40 (21.86)	7	19.71 (19.41)
Control (no-EE)	7	10.29 (4.79)	6	5.00 (3.16)

Note. Grooming time of the facial, dorsal, ventral, and genital areas was summed to produce global grooming time.

Table 4

Mean (Standard Deviation) Rearing Events when both Known and Novel Stimulus Rats were Present on Trial 2 of the Social Preference Task

Condition	Postnatal Day 35 *N*	Postnatal Day 35 *M (SD)*	Postnatal Day 49 *N*	Postnatal Day 49 *M (SD)*
Enriched (EE)	12	3.7 (2.4)	12	5.4 (2.1)
Control (no-EE)	13	5.4 (2.6)	13	4.3 (2.5)
Female	12	4.1 (2.2)	12	5.3 (2.4)
Male	13	5.0 (3.0)	13	4.4 (2.2)

Note. Rearing events were counted over the entire time during a single trial and for each minute of each trial. Rearing events across the entire 3 min trial are presented here.

Figure 1. Two of four different cage set-ups for environmental enrichment are shown. These pictures show "female" cages; male cages were set in exactly the same way as female cages.

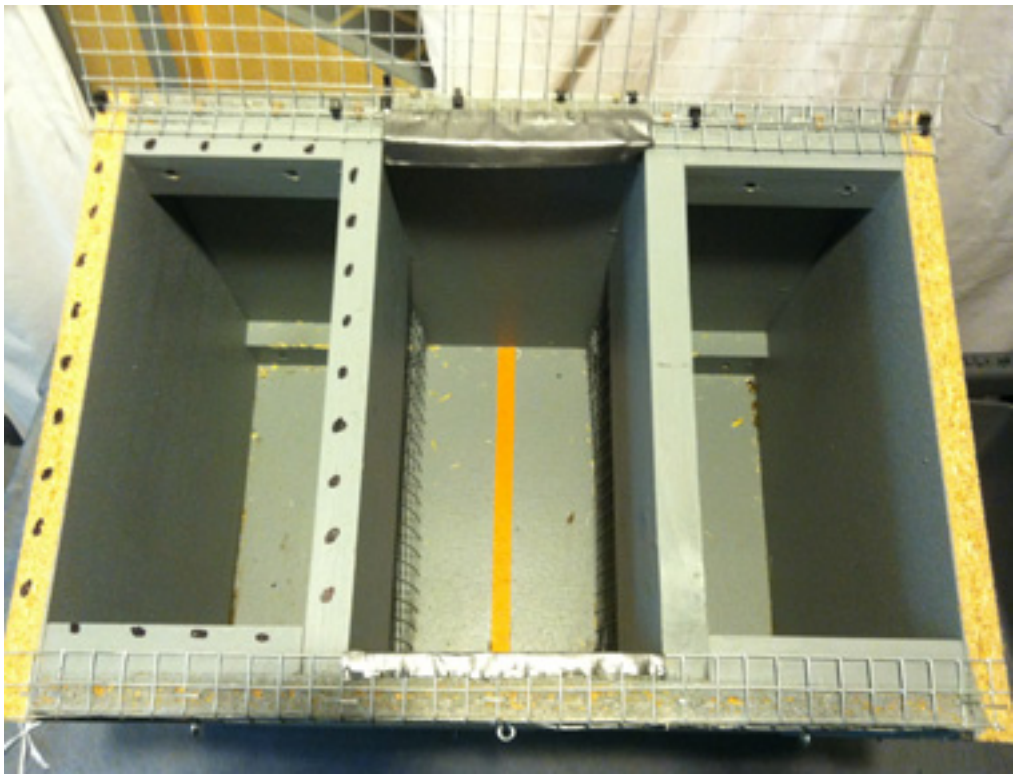

Figure 2. The three chambered social preference testing apparatus is shown. The left most chamber held stimulus rat j1, the middle chamber held the experimental animal, the right most chamber held stimulus rat j2 for the first trial and j3 for the second trial. Only the right most animal was switched between Trials 1 and 2.