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EFFECTS OF TEMPERAMENT AND ADOLESCENT SOCIAL EXPERIENCES ON
ADULT EXPLORATORY BEHAVIOR

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ABSTRACT

Background: The goal of this study is to examine the effects of adolescent social experiences on adult behavior. Peer social interactions are considered to be one of the most crucial components of adolescence because they can produce long-lasting positive or negative behavioral outcomes in adulthood. Several studies have used rodents to model social stress during adolescence because adolescent rodents share similar emotional and social behavior changes with adolescent humans. One of the most prevalent types of adverse social experiences used in rodent studies is social isolation, whereby a rodent is individually housed in the colony room or removed from the colony room altogether for a specified period of time. The current study looked to see if adverse social experiences during adolescence altered exploratory and/or social behavior in adulthood. In addition to examining adolescent social experiences, the current study also examined temperament as a possible factor. Temperament refers to individual differences in behavior due to genetic makeup and may account for inconsistency in adult behavior. We hypothesized that isolated rats and/or inherently inhibited individuals will most likely suffer from the most severe behavior and emotional deficits as adults.

Methods: 53 male Sprague-Dawley rats were weaned from their mothers on post-natal day (PND) 22 and lived in groups of three same-sex siblings until PND 28. On PND 28, all males were randomly assigned to one of three adolescent social conditions—kin groups (KIN), individual housing (IND), or social reorganization groups (SRO). KIN rats were housed in trios of same-sex littermates (N = 18); IND rats were housed individually (N = 18); and SRO rats were housed in trios with unfamiliar non-littermate males (N = 17). Rats remained in the adolescent social conditions until PND 46, at which point they were returned to the original kin trios. Rats underwent an exploration arena test during pre-adolescence (PND 20), mid-adulthood (PND 60), and late adulthood (PND 85). A social challenge was conducted in late adulthood (PND 110).

Results: In mid-adulthood (PND 60), neophobic rats in kin groups and individual housing exhibited higher locomotion compared to neophilic rats that experienced the same social conditions as adolescents. Conversely, neophilic rats that experienced novel social partners during adolescence moved more compared to neophobic rats that had experienced novel social partners during adolescence. Further into adulthood (PND 85), locomotion and inspection no longer significantly differed among rats that experienced different adolescent social conditions. There was a main effect of temperament, though, in which locomotion was greater in neophilic rats than neophobic rats at this later age. Later in adulthood (PND 110), aggression, submission, and inspection did not differ between neophobic and neophilic groups or the 3 adolescent social experience groups.

Conclusion: Adolescent manipulations did not predict behavior based on temperament throughout adulthood, just at one time point. These findings show that individual traits may develop over time but tend to remain stable. The lack of significant differences in social behavior in adulthood suggests that any type of social stress experienced in adolescence may not have long-term detrimental behavioral consequences in adulthood.

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Chapter 1

Introduction

Adolescence is a critical period of transition from childhood to adulthood during which several cognitive, social, and developmental changes occur. In humans, this age spanning roughly 12-18 years includes pubertal maturation, an elevation in novelty-seeking and risk-taking behaviors, as well as an increase in peer-directed social interactions (Spear, 2000). Peer social interactions are considered to be one of the most crucial components of adolescence because they can produce long-lasting psychological and/or physiological effects (Adam et al., 2011; Romeo, 2010; Eiland & Romeo, 2013). Positive adolescent social experiences, such as high levels of social support, can promote welfare, confidence, and overall health in adulthood. Conversely, negative social experiences, such as perceived feelings of loneliness, social exclusion, or bullying can be major sources of stress for adolescents. Adolescent loneliness can also increase the risk of developing adult depression and metabolic conditions associated with cardiovascular disease (Lupien et al., 2009; Goosby et al., 2013; Gladstone et al., 2006). Persistent negative adolescent social experiences may be associated with adverse behavioral and physiological outcomes adulthood; for example, anxiety disorders, which affect 18.1% of the U.S. adult population (Kessler et al., 2005).

Several studies have used rodents to model social stress during adolescence. While rodents' social organization is considerably less complicated than humans, adolescent rodents share similar emotional and social behavior changes with adolescent humans (Spear, 2000). For example, rodents engage in more peer-directed social interactions, exhibit heightened emotionality, and show an increase in novelty-seeking behavior at this stage of development

(Spear, 2000). However, rodents have a much briefer adolescent period than humans that spans from approximately postnatal day (PND) 28 to 46, which makes research development with rodent models more feasible than with humans (Spear, 2000). And, unlike human studies, rodent models allow for experimental manipulation of physiology and/or the environment to determine causal influences of such factors on development.

One of the most prevalent types of social stressors used in rodent studies is social isolation. Social isolation can refer to individually housing a rodent in the colony room or removing the rodent from the colony room altogether for a specified period of time. If rodents are separated from their peers during this sensitive period, they are deprived of the opportunity to learn peer communication techniques and proper social behavior (Varlinskaya & Spear, 2008). Environmental enrichment results in more positive psychological, social, and behavioral outcomes in adulthood (Varlinskaya, Spear, & Spear, 1998; Varlinskaya & Spear, 2008). Depending on the initial age of isolation, type of isolation, degree of sensory deprivation, and duration of isolation, removal from parents and/or congeners can impose severe consequences on these animals (Shabanov et al., 2004). Housing a rodent in a separate cage, away from other animals, has shown to dysregulate stress responses and alter general social behavior (McCormick et al., 2005; Lukkes et al., 2009; Einon, Morgan, & Kibbler, 1978; Toth et al., 2011; Weintraub, Singaravelu, & Bhatnagar, 2010; McCormick, Smith, & Mathews, 2008). While several studies have examined the effects of adolescent social stress on rodents, they have all used various methods and have produced inconsistent results.

Data from studies indicate that adolescence may not be a particularly vulnerable phase and that social stress during this time does not appear to have long-lasting effects; adolescence is viewed more as a period of adaptation (Buwalda et al., 2011). On the contrary, studies have also shown that adolescence is a sensitive period in which any type of social instability can have significant effects on anxiety and depressive behavior in adulthood (McCormick & Green, 2013).

Adolescent Social Stress and Adult Emotionality

Social conditions during adolescence have been shown to affect responses to novelty in adulthood. McCormick et al. (2005) conducted a study in which pre-adolescent (PND 25) rats were housed in same sex pairs until PND 33. On PND 33, rats were randomly assigned to either a no pubertal stress condition or a pubertal stress regimen. The no pubertal stress rodents were housed in same-sex pairs and left undisturbed, while the pubertal stress condition consisted of individually housing rats for 1 hour daily in a room separate from the rest of the colony from PND 33 to 48. Upon daily return to the colony, the stressed rats were placed with a new same-sex, same-treatment cage partner. On PND 48, pubertal stress rats were housed with their original cage partners. On PND 69, rats were tested for locomotor activity in an open field test. The pubertal stress group displayed higher locomotor activity in an open field than the controls, suggesting a greater reaction to novelty in adulthood (McCormick et al., 2005).

Social isolation during adolescence can lead to short-term increases in anxiety-related behavior. For example, rats were either individually housed or put in same-sex groups of 3 from PND 21 to 42. After this period, all rats, including the group-reared rats, were randomly assigned to a new group of three cage partners for two weeks (PND 43-55). To assess anxiety-like behavior, on PND 56, rats were put in an open field test. A conditioned fear behavior test was also conducted in which rats were placed in a chamber for 10 minutes prior to exposure to acoustic tones paired with a foot-shock, and 24 hours later, the rats were placed in the same chamber and exposed to the same acoustic tones without any shocks. Isolated rats displayed an increase in anxiety-like behavior in both types of behavior testing. Specifically, rats exposed to adolescent isolation displayed reduced movement and lower number of entries in the center of the open field and increased conditioned fear behavior (e.g., freezing behavior) compared to group-reared rats (Lukkes et al, 2009). In a separate experiment that also employed an open field test,

rats isolated from PND 25-45 habituated more slowly in an object-filled open field test on PND 45 compared to rats consistently housed in groups of 5 during the same time frame. Over successive trials in the open field test, the social rats exhibited a decline in locomotion and less contact with the objects compared to isolated rats, suggesting a faster acclimation to a novel environment (Einon, Morgan, & Kibbler, 1978).

Lack of stimulation and novelty provided by adolescent social interactions can cause anxiety-like behavior in adulthood as well. Ros-Simó & Valverde (2012) randomly assigned mice to either a social housing condition (5 cage partners), an enriched housing condition (novel objects and 10 cage partners), or an isolated housing condition (without objects or cage partners) from PND 21 to 70. On PND 71-73, locomotor activity was assessed by measuring the number of horizontal and vertical movements in an activity box. On PND 74, anxiety-like behavior was measured in an elevated plus-maze and on PND 75 a tail suspension test was conducted to evaluate depression-like behavior (mice were suspended by tail for 6 minutes and total time of immobility was recorded). Isolation was found to enhance locomotion in the locomotor activity test, increase anxiety-like behavior in the elevated plus-maze (i.e., increased frequency of entries into arms with vertical walls vs. unprotected edges), and increase mobility in the tail suspension test, indicating that lack of enrichment in adolescence can lead to anxious behavior in adulthood (Ros-Simó & Valverde, 2012).

In a study by McCormick, Smith, & Mathews (2008), rats were assigned to a chronic adolescent social stress condition, no-stress control condition, or acute stress control condition from PND 30 to 45. Rats in the chronic stress group were isolated from colony cage mates for 1 hour in a room separate from the colony on each day during the adolescent manipulation. Rats in the acute stress group were isolated for 1 hour in a room separate from the colony only on PND 45 and rats in the no-stress control group were left undisturbed. Rats were then tested in an elevated plus-maze (EPM) on both PND 45 and PND 70. There were no significant immediate

effects of stress (i.e., at PND 45); however, chronically-stressed rats showed an increase in anxiety-like behavior on PND 70 (McCormick, Smith, & Matthews, 2008). Another study also used an EPM to study the long-term effects of adolescent stress. Rats were either individually housed or housed in same-sex groups of 3-4 from PND 30 to 50. All rats were re-housed in same-sex same-treatment groups of 2-3 from PND 50 until they were tested on the EPM on PND 80 or 81 for 10 minutes. Males isolated in adolescence spent more time in the unprotected open arms and less time in the walled closed arms of the maze compared to control males, suggesting a reduced state of anxiety in adulthood (Weintraub, Singaravelu, & Bhatnagar, 2010).

The different outcomes from these studies suggest that length of adolescent social manipulation, timing of manipulation, and timing of outcome measures can result in different emotional behaviors exhibited in adulthood. Isolated males in the Weintraub, Singaravelu, & Bhatnagar (2010) study were isolated for 20 days (PND 30-50) and showed reduced anxiety-like behavior in the EPM as adults. However isolated rats in the Ros-Simó & Valverde (2012) study that were isolated for much longer (PND 21-70) showed an increase in anxiety-like behavior in the EPM as adults. Similarly, isolated rats in the study by McCormick et al. (2005) had higher levels of locomotion after being stressed for 15 days (PND 33-48), while isolated rats in the Lukkes et al. (2009) study were isolated for a longer period of time (PND 21-42) and showed reduced movement in an open field test. Timing of adolescent manipulation and/or outcome measure also differed between these studies. Studies by Lukkes et al. (2009), Einon, Morgan, & Kibbler (1978), and Ros-Simó & Valverde (2012) began experimental manipulations before the adolescent period began (PND 21, PND 25, and PND 21, respectively). In contrast, McCormick et al. (2005), McCormick, Smith, & Matthews (2008), and Weintraub, Singaravelu, & Bhatnagar (2010) implemented the manipulation in the beginning of the adolescent period (PND 33, PND 30, and PND 30, respectively). Some studies measured emotional outcomes immediately after experimental manipulation (Einon, Morgan, & Kibbler, 1978; Ros-Simó & Valverde, 2012;

McCormick, Smith, & Matthews, 2008). Other studies waited a longer amount of time before conducting tests in adulthood (McCormick et al., 2005; Lukkes et al., 2009; Weintraub, Singaravelu, & Bhatnagar, 2010).

Adolescent Social Stress and Adult Social Behavior

Some studies have used adult social behavior outcomes as a way to assess the effects of adolescent social stress on adult behavior. A study weaned rats on PND 21 and housed them either individually or in groups of four until PND 76, when all animals were housed in individual cages. To measure adult aggressive and submissive behavior toward a conspecific, a resident-intruder test was conducted on PND 82, in which intruder rats were placed in resident rat cages for 20 minutes and behavior of the residents was recorded. Rats that had been continuously isolated interacted with intruder rats more frequently, transitioned their behaviors at a more rapid rate, were less likely to signal attack intentions, and more likely to attack the intruders from abnormal positions (e.g., submissive or defensive posture) compared to socially housed rats (Toth et al., 2011). These results suggest that isolated rats are more likely to display behavioral effects of social stress compared to socially housed rats.

In a similar study, Kercmar et al. (2010) weaned mice at PND 21 and randomly assigned them to one of three groups for PND 21-80: social group (housed in same-sex groups of three), isolated group (individually housed), or isolated/social group (temporarily isolated for first 30 days and then put into same-sex trios). On PND 80, mice experienced a social recognition test in which an ovariectomized female was put into the cage for 1 minute with the male, removed for 9 minutes, and then placed back into the cage and removed in the same intervals 8 more times. On a final trial, a new partner was introduced. During each trial, duration of sniffing by the test subject was recorded. Time spent sniffing familiar females linearly decreased over time for social group

mice and reverted to initial sniffing time with the arrival of an unfamiliar female on the last trial. Male mice that had been isolate housed showed little change in time spent sniffing the same female mouse across all trials, suggesting a deficit in social learning. The isolated/social group mice displayed a social recognition pattern of behavior (i.e., spent less time sniffing familiar females compared to unfamiliar females) however, compared to social group mice, there was no change in time spent sniffing the familiar females across trials.

Both studies by Kercmar et al. (2010) and Toth et al. (2011) showed that either long-term or transient social isolation during development can impact social behavior in adulthood. Each experimental manipulation lasted for the same amount of time (PND 21- PND 76/80) and demonstrated that whether a subject is socially deprived just during adolescence or during both adolescence and adulthood, they will display deficits in social behavior as adults. The studies differed, however, in that one began behavior testing immediately after experimental manipulation and the other individually housed subjects for 6 days before testing. Even though the studies did not truly isolate animals from the colony and used individual housing as a social stressor, the adolescent manipulation lasted much longer than other studies. The outcomes revealed that social enrichment as an adolescent is important for normal social interactions with peers as well as social learning and habituation as an adult.

While several studies have examined the influence of diminished adolescent social experience on behavior and/or emotionality in adulthood, few have tested the influence of diminished adolescent social experience on social behavior in particular. The current study looked at the potential long-term consequences of adolescent social experiences on adult social abilities. The current study aimed to see if the effects revealed in previous studies last into adulthood. Additionally, no prior studies have compared how novel social experiences during adolescence compare to only familiar social experiences. Two types of social conditions were implemented in the experimental manipulation to see if a novel social environment is considered

to be more stressful than a familiar one. Lastly, the current study aimed to see if returning rodents to kin cage partners after adolescent social conditions would dampen any possible effects of social stress on adult behavior.

Temperament

The outcomes as described above may be due to adolescent social manipulations, but they may also be influenced by innate differences in behavior. There tends to be individuals who display consistent differences in behavior. For example, some may react fearfully to novelty ("neophobic") whereas other may seem to crave or like new experiences ("neophilic"). Individual differences in temperament arise from differences in genetic makeup and are shaped by environmental experiences during early childhood and adolescence (Sachser, Kaiser, & Hennessy, 2013). In humans, highly inhibited individuals (those who tend to resist or withdraw from novelty) have very different social experiences than highly exuberant ones. Exuberant people enjoy risky activities and highly stimulating social interactions. Inhibited people, however, are less interactive with their peers and are more likely to be socially neglected; they do not experience as much peer conflict or aggression (Tarullo, Mliner, & Gunnar, 2011). Different temperaments in early childhood and adolescence may predict behavior in adulthood. For example, behavioral inhibition in childhood and adolescence may predict substance use later in life; individuals who shy away from social interactions with peers may be more likely to abuse substances in adulthood as a means to approach novel social situations (Lahat et al., 2012). Conversely, behavioral inhibition (but not social withdrawal specifically) may serve as a protective factor against future deviant behavior compared to peers who display more exuberant or aggressive behavior (Kerr et al., 1997). The current study considers the possibility of an interaction effect between temperament and adult social behavior.

The degree to which temperament accounts for behavior in adulthood is uncertain (Hirshfeld-Becker et al., 2008). Researchers debate as to whether or not individual traits based on temperament are stable across the lifetime. Some studies have shown that innate differences in temperament can shape adult behavior and increase the likelihood of habituation to novelty (Fox & Millam, 2004; Roberts, Caspi, & Moffitt, 2001; Rödel & Meyer, 2011; Tang et al., 2012). Studies show that traits are most consistent in mid-life and most flexible during development (Roberts & DelVecchio, 2000). Other research has indicated that individual traits are not as stable over time, and that early-life experiences based on temperament may not always predict behavior as an adult (Gracceva, Koolhaas, and Groothuis, 2011). Nevertheless, temperament must be considered when examining adolescent stress on adulthood behavior, as it may account for inconsistency in experimental results. For instance, certain temperaments may be more resilient or susceptible to social experiences than others, and this can determine to what extent adverse social experiences in adolescence account for behavioral outcomes in adulthood.

The present study assessed the effects of adolescent social experiences on adult social behavior. Unlike several prior studies that have used rodent models, this study compared not only social deprivation outcomes to social enrichment outcomes, but looked to see if there was a difference in adult behavior among rats who socialized with kin vs. rats who socialized with unrelated conspecifics. Given the outcomes from previous experiments, we hypothesize that an isolated rat will most likely suffer from the most severe behavior and emotional deficits as adults. The experiment was ultimately conducted in order to shed more light on the following questions: (1) Does individual housing alter adult emotionality and social behavior? (2) Does exposure to novel social partners in adolescence predict enhanced habituation to novelty and higher levels of social interactions in adulthood compared to exposure to kin cage partners? (3) Are some individuals more susceptible or resilient to these influences than others due to temperament?

Chapter 2

Methods

Subjects

There were 53 male Sprague-Dawley rats from 15 litters (breeder rats were purchased from Charles River Breeding Laboratories) and each was ear notched for individual identification. Rats were housed in solid bottom plastic cages (43.5 x 23.5 x 20.5 cm) in a colony room at 20 ºC with ~50% humidity. Food and water were available *ad libitum*. Rats were maintained on a 14L:10D lighting schedule with lights on at 2000 h (Central Standard Time, CST). Cages were cleaned twice a week by trained animal facility personnel and rats were gently handled three times a week. Males were weaned from their mothers on post-natal day (PND) 22 and lived in groups of three same-sex siblings until PND 28. All methods were approved by the University of Chicago Institutional Animal Care and Use Committee (IACUC) and adhere to the Guide for the Care and Use of Laboratory Animals.

Overall Study Design

Adolescent social conditions began on PND 28 and lasted until PND 46 because PND 28-46 has been established as the full extent of adolescence in rodents during which individual housing and/or social isolation produce long-term effects on behavior (Einon & Morgan, 1977; Einon, Morgan, & Kibbler, 1978; Spear, 2000; McCormick & Green, 2013). Rats underwent the exploration arena test during pre-adolescence (PND 20), mid-adulthood (PND 60), and late adulthood (PND 85) to determine if personality traits due to temperament remained stable across

the lifespan. A social challenge was conducted in late adulthood (PND 110) to see if stress during

adolescence had enduring effects on adult behavior (see Figure 1).
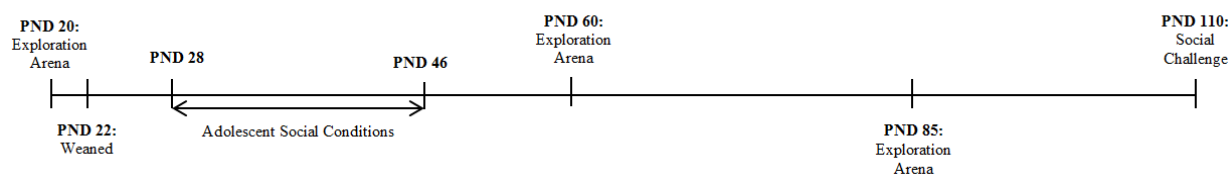
Figure 1. Experimental Procedure**.**

Temperament Measures

On PND 20, male rats were tested for locomotion in an exploration arena (described

below). Mean locomotion scores from the two trials in the arena were used to determine

temperament. Males with the highest locomotion relative to the litter mean were neophilic. The

rat with the median score of the litter was classified as the neophobic subject, and a rat was put as

the third cage mate for the neophobic and neophilic rats. Unresponsive rats were not included in

analyses.

Adolescent Social Conditions

On PND 28, all males were randomly assigned to one of three adolescent social

conditions—kin groups (KIN), individual housing (IND), or social reorganization groups (SRO).

KIN rats were housed in trios of same-sex littermates (N = 18); IND rats were housed

individually (N = 18); and SRO rats were housed in trios with unfamiliar non-littermate males (N

= 17). Rats remained in the adolescent social conditions until PND 46, at which point they were

returned to the original kin trios. Males stayed in their kin groups until PND 110, at which point

each rat was tested in a social challenge test and individually housed immediately after the test as part of a larger study on temperament and health.

Exploration Arena

On PND 20, 60, and 85, subjects were assessed in a novel exploration arena in a dimly lit non-colony room 5-8 h into the dark phase. The arena on PND 20 measured 92 x 92 x 23 cm and the arena on PND 60 and 85 measured 122 x 122 x 46 cm. The arena had a Plexiglas cover superimposed with a 3 x 3 grid. A small object (bowl, brick, tunnel, or empty food hopper) was placed in three of the four corners. Objects were replaced at the beginning of each trial with new ones to guarantee a novel experience for the test subject. The floor of the arena was covered with clean wood chips that were sprinkled with some bedding soaked with urine by all the colony animals. If a rat defecated, feces were removed with no other cleaning. Males were placed in a ceramic bowl (5-cm walls) and lowered into the empty corner of the arena. Each rat was tested for 5 minutes, during which time their behavior was recorded to measure the animal's locomotion and inspection. Locomotion was defined as the number of lines crossed on the 3 x 3 grid with 4 paws. Inspection was defined as the number of times a rat touched an object with 2 paws or nose or climbed on and/or entered an object. After testing, the rat was immediately returned to the colony room.

Social Challenge Arena

The purpose of the social challenge was to determine each rat's willingness to interact with novel social partners. Males were placed in a large novel arena with two other novel males for 1-hour. Three males were tested in the arena at once, each having been randomly selected

from different litters with no history of prior social interaction. Social behavior was recorded for

1 hour. Behavior was videotaped to measure aggression initiated, aggression received, submission

initiated, submission received, and inspect conspecific. All of these data were frequency counts.

Initiated behaviors were those started and/or facilitated by the test subject. Received behaviors

were those initiated or imposed upon by other rats towards the test subject. Aggressive behaviors

included threats/thrusts (any type of head or forebody movement toward conspecific), attacks

(rapid approach carried on over back of conspecific and subject's head came in contact with far

flank), bites (teeth gripped skin of conspecific), chases, full aggressive postures (subject

orientated himself at right-angles to and over conspecific's body), and aggressive grooming

(subject used teeth to pull fur vigorously on conspecific, localized to shoulder region).

Submissive behaviors included behavior displaying defeat, such as a full submissive posture

(subject lay flat on its back) and crouching down. Inspection behaviors included investigate

(exploration of conspecific except for facial and ano-genital regions), sniff (exploration of

conspecific ano-genital regions), and nosing (close contact between noses of subject and

conspecific) (Grant & Mackintosh, 1963). After testing, rats were immediately returned to the

colony room and individually housed as part of a larger study on temperament and health.

Statistical Analyses

Pearson's correlations of all the dependent variables were calculated, and partial

correlations were calculated with litter number as the covariate. To determine if temperament and

adolescent social condition affected behavior in the exploration arena, univariate analyses of

variance (ANOVA) were conducted with temperament and adolescent social conditions as

independent variables and locomotion on PND 20, inspection on PND 20, locomotion on PND

60, inspection on PND 60, locomotion on PND 85, and inspection on PND 85 as dependent

variables. Litter means were included as covariates (i.e., litter mean of inspection on PND 60 if the dependent variable was inspection on PND 60).

A Kolmogorov-Smirnov test was conducted to see if the data were normally distributed. To test the hypothesis that adolescent social condition affected adult social behavior, a 2 x 3 (temperament x adolescent social condition) univariate ANOVA and a Bonferroni post-hoc test were conducted with adolescent social conditions and temperament as independent variables and frequency of inspect conspecific as the dependent variable. The Bonferroni post-hoc test was done to correct for multiple comparisons with a more stringent p-value. Frequency of aggression and frequency of submission were analyzed using a non-parametric Independent-Samples Kruskal-Wallis Test because the variables were not normally distributed. Litter means were used as covariates to control for any litter effect.

Some social groups in the social challenge test were more interactive than other trios. Additionally, rates of individual rat behavior were of more interest in the study than overall group behavior. Thus, group effects were controlled for by calculating percent aggression, percent submission, and percent inspect conspecific. Percent aggression, percent submission, and percent inspect conspecific were calculated by dividing the total number of behaviors exhibited by all rats in the arena from the frequency of aggressive, submissive, or inspection behaviors initiated and received by the subject. Three IND rats were not included in statistical analyses because data were lost due to equipment malfunction. To determine if adolescent social experiences predicted adult social behavior, a univariate ANOVA and a Tukey post-hoc test were conducted with adolescent social conditions as factors and percent aggression and percent inspect conspecific as dependent variables. Percent submission was analyzed using a non-parametric Independent-Samples Kruskal-Wallis Test.

Chapter 3

Results

Exploration Arena

There was a moderate positive correlation between locomotion on PND 20 and PND 85, $r_{51} = .33$, $p < .05$. Locomotion on PND 60 and 85 were positively correlated, $r_{50} = .73$, $p < .001$. There was a strong positive relationship between PND 20 locomotion and PND 20 inspection, $r_{44} = .79$, $p < .001$. There was a moderate positive correlation between PND 20 inspection and PND 85 locomotion, $r_{44} = .31$, $p < .05$. On PND 60, locomotion and inspection were strongly positively correlated, $r_{50} = .91$, $p < .001$. On PND 85, locomotion and inspection were positively correlated, $r_{51} = .92$, $p < .001$. Inspection on PND 60 and PND 85 were positively correlated, $r_{51} = .68$, $p < .001$. Partial correlation analyses revealed that controlling for litter did not change the relationship between locomotion and inspection at any age.

PND 20

Locomotion was approximately twice as high in neophilic rats compared to neophobic rats. Neophilic rats exhibited significantly greater inspection of novel objects compared to neophobic rats, $F_{1,39} = 7.50$, $p < .01$ (see Figures 2 and 3). Adolescent social condition was not associated with locomotion or inspection, $F_{2,46} = .19$ and $F_{2,39} = 1.02$, p's $> .05$. There was no significant interaction effect of temperament and adolescent social condition on locomotion or inspection, $F_{2,46} = 1.51$ and $F_{2,39} = .99$, p's $> .05$ (see Figure 3).
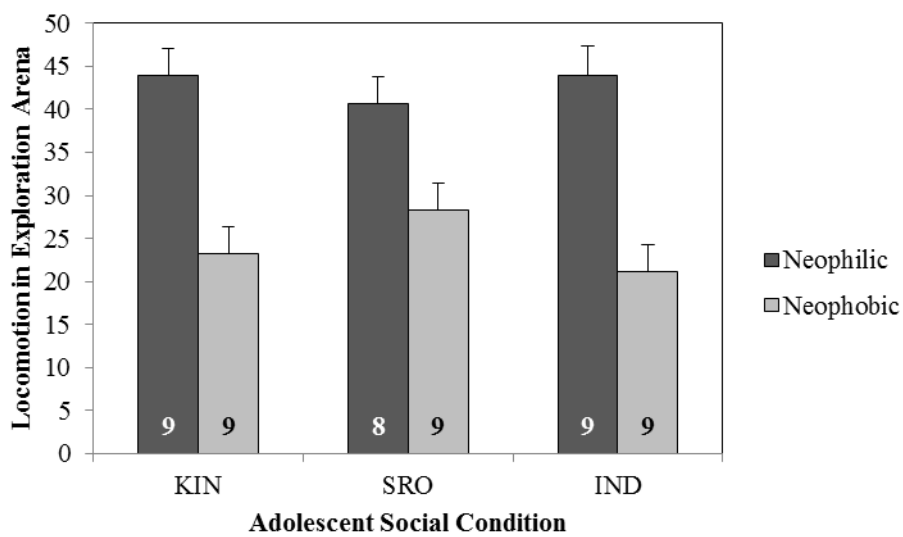
Figure 2. Estimated marginal means for the frequency of locomotion exhibited by male rats in the exploration arena on PND 20 (N = 53). Error bars indicate S.E.M.
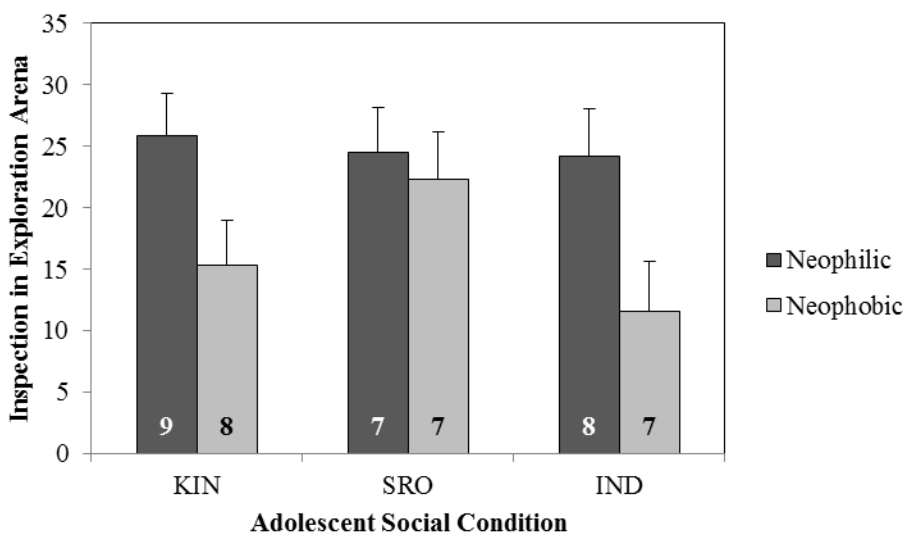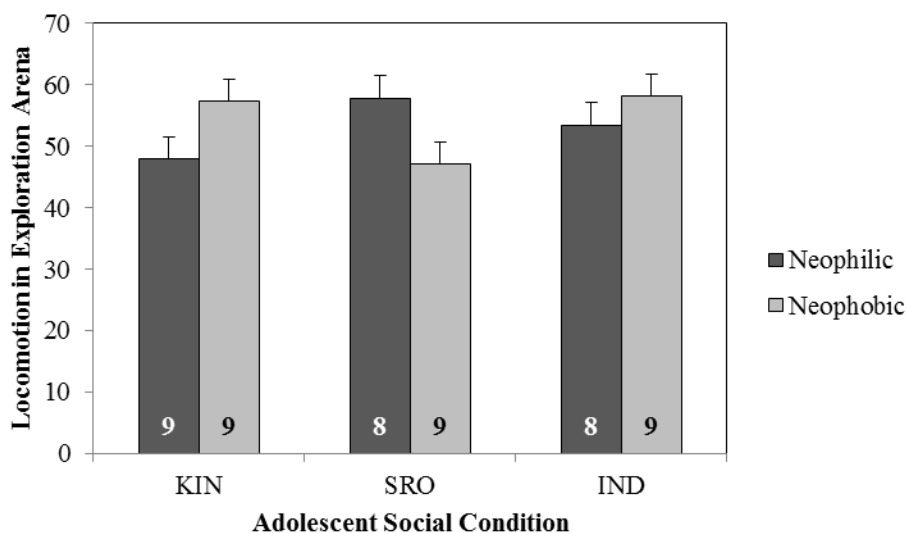

Figure 3. Estimated marginal means for the frequency of inspection exhibited by male rats in the exploration arena on PND 20 (N = 46). Error bars indicate S.E.M.
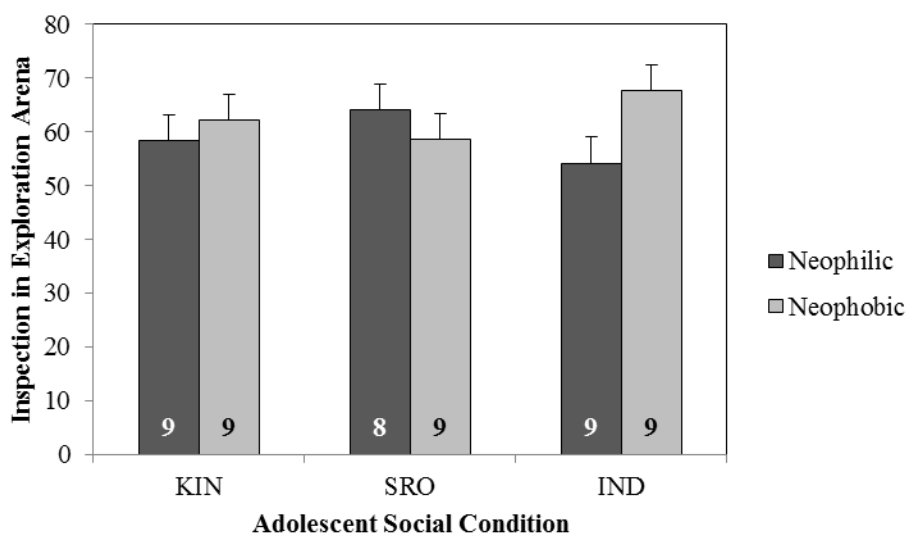
PND 60

Locomotion and inspection did not significantly differ between neophilic and neophobic

groups or between adolescent social condition groups, $F_{1,45} = .16$, $F_{2,45} = .52$, $F_{1,46} = 1.04$, $F_{2,46} = .03$ p's $> .05$ (see Figure 5). There was a statistically significant interaction between temperament and adolescent social condition on locomotion, $F_{2,45} = 4.33$, $p < .05$ (see Figure 4).
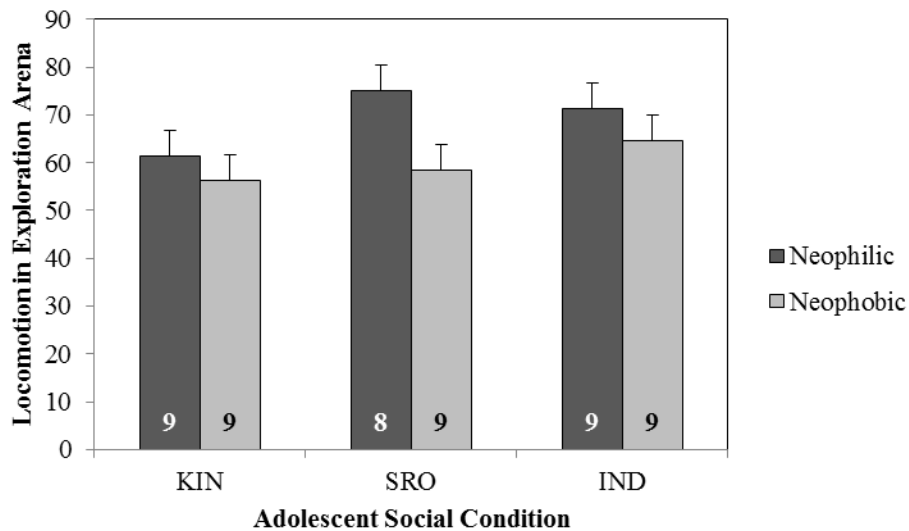

Figure 4. Estimated marginal means for the frequency of locomotion exhibited by male rats in the exploration arena on PND 60 (N = 52). Error bars indicate S.E.M.

Figure 5. Estimated marginal means for the frequency of inspection exhibited by male rats in the exploration arena on PND 60 (N = 53). Error bars indicate S.E.M.
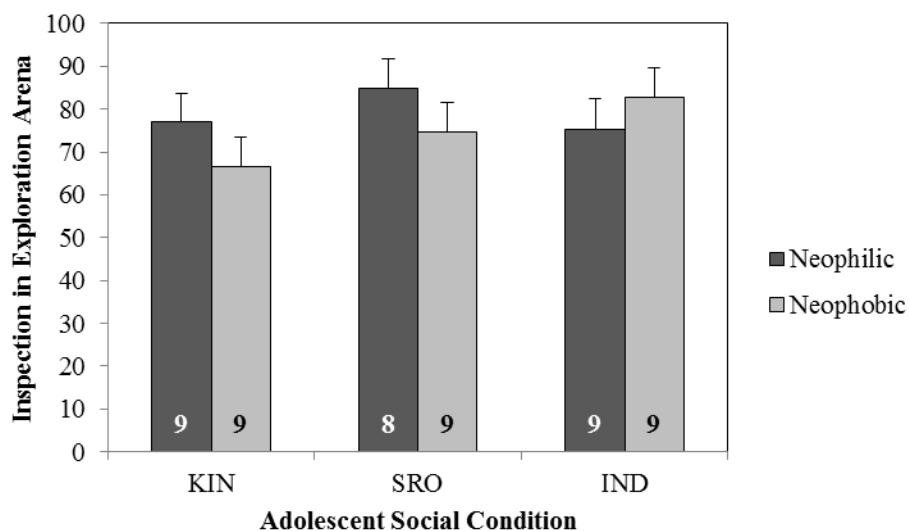
PND 85

Locomotion was significantly greater in neophilic group than the neophobic group, $F_{1,46} =$ 4.64, $p < .05$. There was no difference in locomotion between control and isolation groups, control and social reorganization groups, or between isolation and social reorganization groups, $p > .05$. There was no significant interaction between temperament and adolescent social condition on locomotion, $p > .05$ (see Figure 6). Total inspection was the same for both neophilic and neophobic groups, $p > .05$, and did not differ between adolescent social condition groups, $F_{1,46} = .58$, $F_{2,46} = .83$, $p > .05$ (see Figure 7).

Figure 6. Estimated marginal means for the frequency of locomotion exhibited by male rats in the exploration arena on PND 85 (N = 53). Error bars indicate S.E.M.
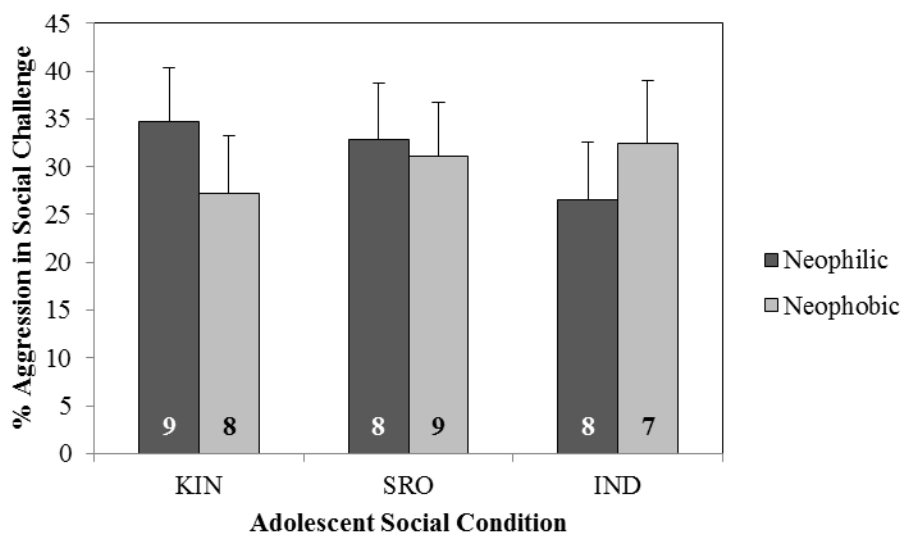
Figure 7. Estimated marginal means for the frequency of inspection exhibited by male rats in the exploration arena on PND 85 (N = 53). Error bars indicate S.E.M.

Social Challenge Arena

There was no significant effect of temperament or adolescent social condition on adult rats' frequency of aggression, submission, or inspection of conspecifics in the social challenge test at PND 110; $F_{2,43} = .16$, $F_{2,43} = 2.09$, $F_{2,43} = .39$, p's > .05. The same results emerged when percent aggression, submission, and inspection were used instead of frequency; $F_{2,43} = .61$, $F_{2,43} = 1.13$, $F_{2,43} = .23$, p's > .05 (see Figures 8, 9, 10).

Figure 8. Estimated marginal means for percent aggression exhibited by male rats in the social challenge arena on PND 110 (N = 49). Error bars indicate S.E.M.
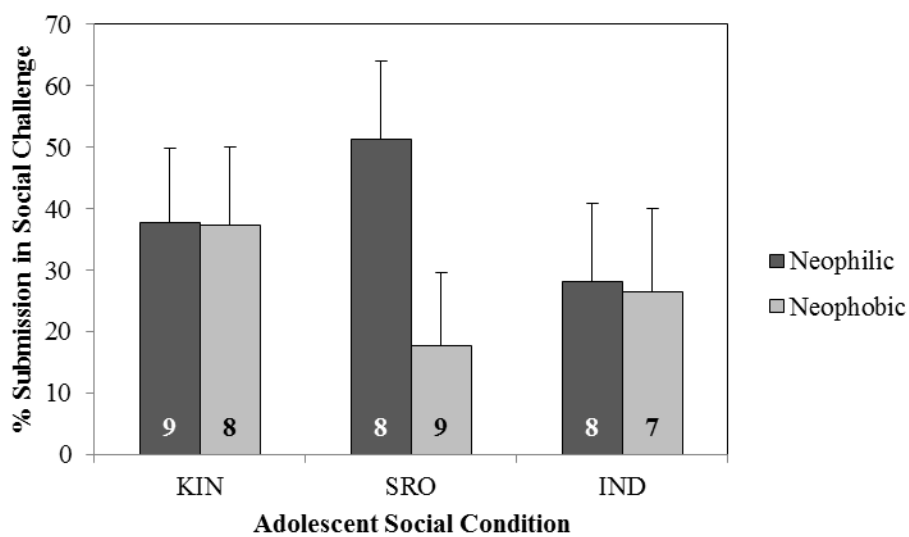
Figure 9. Estimated marginal means for percent submission exhibited by male rats in the social challenge arena on PND 110 (N = 49). Error bars indicate S.E.M.
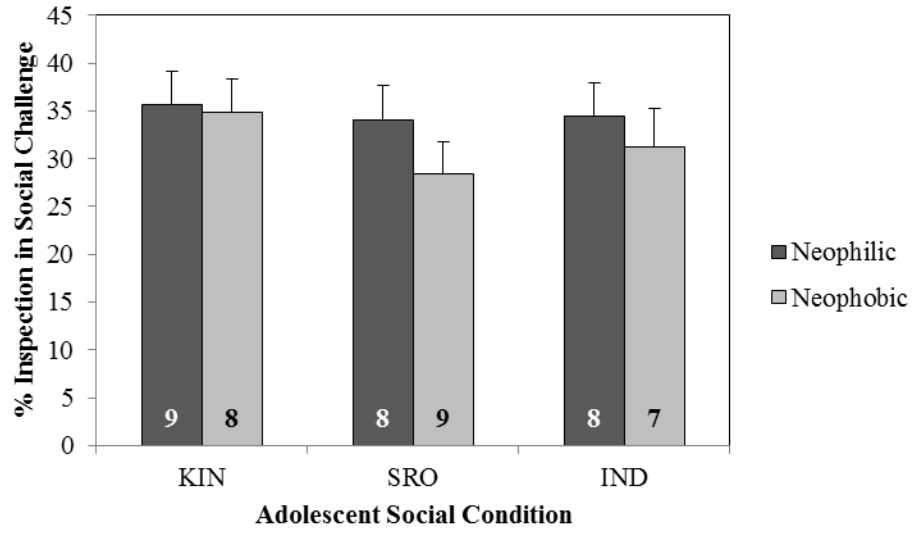
Figure 10. Estimated marginal means for percent inspection exhibited by male rats in the social challenge arena on PND 110 (N = 49). Error bars indicate S.E.M.

Chapter 4

Discussion

The results from the current study indicate that social stress in adolescence may have a transient effect on locomotor responses in adulthood. In mid-adulthood (PND 60), neophobic rats in kin groups and individual housing exhibited higher locomotion compared to neophilic rats that experienced the same social conditions as adolescents. Conversely, neophilic rats that experienced novel social partners during adolescence moved more compared to neophobic rats that had experienced novel social partners during adolescence. Further into adulthood (PND 85), locomotion and inspection no longer significantly differed among rats that experienced different adolescent social conditions. There was a main effect of temperament, though, in which locomotion was greater in neophilic rats than neophobic rats at this later age. Later in adulthood (PND 110), aggression, submission, and inspection did not differ between neophobic and neophilic groups or the 3 adolescent social experience groups.

Effects of Adolescent Social Experiences on Adult Behavior

Similar to several prior studies, the current study assessed the effects of adolescent social conditions on behavior by measuring locomotor activity in adulthood. Results from previous experiments are mixed. Studies have shown that adverse adolescent social conditions (individual housing or isolation) increase locomotor activity in adulthood (McCormick et al., 2005; Einon, Morgan, & Kibbler, 1978; Ros-Simó & Valverde, 2012). Others have shown that adverse social conditions decrease locomotive behavior in adulthood (Lukkes et al., 2009). The current study found that rats exhibit higher or lower levels of locomotion in mid-adulthood, depending on their

temperament and adolescent social conditions, and that these differences do not continue into late adulthood. The current study also found that adolescent social conditions do not predict social behavior in late adulthood. These results contradict the previous findings that individual housing predicts alterations in adult social behavior and deficits in social learning (Toth et al., 2011; Kercmar et al., 2010).

In the current study, the lower levels of locomotion among neophilic rats exposed to siblings during adolescence may be a result of lack of novelty during adolescence. While these rats had cage partners and had the opportunity to interact with conspecifics, kin cage partners are siblings and may not provide the same amount of enrichment as unrelated new cage partners. The neophobic rats in the same condition may have had a more enriching social experience during adolescence because they lived with familiar littermates. They may not have considered the environment to be socially adverse because the kin cage partners were related. Even though the neophobic rats were considered to be more apprehensive towards approaching novelty, they exhibited higher levels of adult exploratory behavior, perhaps because their adolescent social condition was not extremely unfavorable.

In mid-adulthood, there was no difference in temperament groups on locomotion in individually housed rats, suggesting that individual housing was neither enriching nor harmful for the neophobic and neophilic rats. Unlike kin groups and novel social groups, lack of cage mates may negate any inherent behavioral tendencies in adulthood.

Neophilic rats exposed to novel social partners moved more than their neophobic counterparts exposed to the same adolescent experience. Contrary to the kin groups, social reorganization provides neophilic rats with the opportunity to socialize with new cage partners during adolescence. For neophilic individuals, a novel social environment in adolescence is enriching, as they are able to continuously interact with novelty and explore new objects. The socially reorganized neophobic rats, however, may find this condition to be adverse because they

naturally fear novelty, including unfamiliar cage partners. The condition may be socially debilitating and make them fearful of exploring novelty in mid-adulthood.

The fact that these differences across social groups disappeared in late adulthood suggests that adolescence may not be a sensitive period, especially if the social condition is relatively short-term, and that behavior or experiences in adolescence do not necessarily predict social and exploration behavior in adulthood.

Temperament across the Lifespan

The current study found that temperament prior to any sort of adolescent experience may predict an individual's particular response to a particular adolescent experience. For example, we found that neophobic rats that experience a novel or social environment in adolescence (social reorganization) may have an elevated fear response in novel situations in adulthood, whereas neophobic rats that experience a familiar or comfortable environment in adolescence (kin groups or individual housing) will exhibit less fearful behavior to novelty. On the contrary, neophilic rats that experience a novel social environment in adolescence will move more in an adult novel experience compared to neophilic rats that experienced a familiar or solitary environment in adolescence. However, neither neophilic nor neophobic individuals, though, may show long-term susceptibility or resilience to adolescent social conditions. In the current study, adolescent manipulations did not predict behavior based on temperament throughout adulthood, just at one time point. These findings show that individual traits may develop over time but tend to remain stable.

Differences in Methodology

Differences in results between the current study and previous studies may be attributed to differences in methodology. Compared to previous studies that began adolescent social stressors before the start of adolescence in a novel social situation (i.e., PND 21-25), the current study implemented adolescent social conditions on PND 28, the first day of the adolescent period according to Spear (2000) (Lukkes et al., 2009; Einon, Morgan & Kibbler, 1978; Ros-Simó & Valverde, 2012). Additionally, the current study individually housed rats for only 18 days (PND 28-46) instead of longer periods of time used in other studies (e.g., PND 21-76 or PND 21-80) (Kercmar et al, 2010; Toth et al, 2011). The current study individually housed rats whereas previous studies with similar experimental manipulation length completely isolated rodents from the colony (McCormick et al, 2005; Einon, Morgan, & Kibbler, 1978; McCormick, Smith, & Matthews, 2008; Weintraub, Singaravelu, & Bhatnagar, 2010).

The current study returned all rats to familiar kin groups immediately after the adolescent social manipulations until behavior testing in adulthood whereas previous studies returned rodents to novel social conditions or individual housing immediately after adolescent conditions until testing (Lukkes et al., 2009; Weintraub, Singaravelu, & Bhatnagar, 2010). Returning all rats to kin groups after adolescent social conditions may have negated any effects of adolescent social experiences. If the study individually housed rodents before testing, there may have been significant differences in behavior between social groups.

The design of the social challenge may have played a role in lack of significant findings as well. This study placed three rats in the arena at the same time before recording behavior. No rodent was in the arena before any other rodent. Previous studies measured social behavior in an individual rat by placing an intruder and/or unfamiliar rodent in the arena after the test subject was placed (Toth et al., 2011; Kercmar et al., 2010). The delayed arrival of a conspecific in prior

studies may have explained the behavior observed by the subjects. Subjects in this study, specifically individually housed rats, may have reacted differently in the social challenge test if they acclimated to the environment alone before novel rodents were placed in the arena.

Limitations and Implications

The study had several limitations. Adolescent social stressors were only implemented for two weeks. The short duration of the experimental manipulation could possibly explain the lack of significant findings on PND 85 and 110. A longer period of adolescent social stress may have produced long-term effects on behavior in late adulthood. The social stress paradigm was also not as stressful as in previous studies because rats were not completely isolated from the colony room. Complete removal from the colony room may have been more stressful for the rats and may have produced significant alterations in behavior in adulthood. Another limitation of the study could be the environment in which temperament was examined. Temperament may more measurable in the field than in the lab. Behavior is found to be more repeatable in the field, and greater environmental variance within the field versus a laboratory may allow for rats to better express their individual differences in behavior (Bell, Hankison, & Laskowski, 2009). Social behavior is also very hard to measure. The study measured social behavior of individual rats; however, social behavior is often dependent on the dynamic and behaviors of conspecifics. Focusing on the social behavior of the individual and not examining the social behavior of the group may provide an inaccurate representation of behavior. We tried to adjust our measures to address this issue (i.e., using percent scores), however this may not have controlled for all the variance seen among social groups used during testing.

The results of the study contribute to our understanding of social experience during adolescence. The current study did not produce findings similar to the previous studies

mentioned; however, the lack of significant differences in social behavior suggests that adolescence may not be a developmental phase in which effects of stress continue into adulthood. Social experience is not the only source of stress in adolescence, and other components such as education and the presence of supportive parents may serve as protective factors against adverse social experiences (Burt & Paysnick, 2012; Smith et al., 2013). Social stress experienced in adolescence may not have long-term detrimental behavioral consequences in adulthood if other positive environmental factors promote resilience.

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