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Social interactions in adolescent and adult Sprague–Dawley rats: Impact of social deprivation and test context familiarity

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Abstract

Interactions with peers become particularly important during adolescence, and age differences in social interactions have been successfully modeled in rats. To determine the impact of social deprivation on social interactions under anxiogenic (unfamiliar) or non-anxiogenic (familiar) test circumstances during ontogeny, the present study used a modified social interaction test to assess the effects of 5 days of social isolation or group housing on different components of social behavior in early [postnatal day (P) 28], mid (P35), or late (P42) adolescent and adult (P70) male and female Sprague–Dawley rats. As expected, testing in an unfamiliar environment suppressed social interactions regardless of age, housing, and sex. Social deprivation drastically enhanced all forms of social behavior in P28 animals regardless of test situation, whereas depriving older animals of social interactions had more modest effects and was restricted predominantly to play fighting—an adolescent-characteristic form of social interactions. Social investigation—more adult-typical form of social behavior was relatively resistant to isolation-induced enhancement and was elevated in early adolescent isolates only. These findings confirm that different forms of social behavior are differentially sensitive to social deprivation across ontogeny.

Keywords

Social behavior; Social interaction test; Social deprivation; Adolescence

1. Introduction

Interactions with peers during adolescence are thought to be of principal importance for social development in human adolescents, with individuals spending more time interacting with peers during adolescence than at any other developmental period [10,16]. Similarly, adolescent rats demonstrate higher levels of social behavior than younger and older animals, with these interactions being essential for developing the ability to express and understand intraspecific communication signals [11,17,30,34,35]. Interactions with peers provide a significant source of positive experiences for human adolescents [15,16] and, even in a

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simple model of adolescence in the rat, have been shown to be more rewarding for adolescents than for their more mature counterparts [4].

Social behavior of adolescent and adult rats is sensitive to environmental factors, with, for example, social interactions being notably lower when animals are placed together in an unfamiliar relative to a familiar testing chamber [7,27,37]. An unfamiliar testing situation is traditionally viewed as an anxiogenic condition, and the decrease of social interactions under this condition has been used as an animal model of anxiety [5,6,7,8]. Such environment-induced social inhibition has been reported to differ as a function of age and sex, with young adolescent animals and adult females less affected by an unfamiliar test situation than older adolescents and adult males. For instance, male rats tested at postnatal day (P) 35 and P60 have been reported to demonstrate a reliable decrease in time spent in social interactions under unfamiliar test circumstances, whereas P28 males showed no suppression of social interactions under these test conditions [27]. In contrast, other researchers observed environment-dependent changes in social behavior as early as P21 when play fighting was assessed separately from other types of social behavior [36]. In that study, play fighting, but not social investigation and contact behavior, was suppressed by an unfamiliar environment in P21 male rats. According to Johnston and File [14], adult female rats, in contrast to their male counterparts and adolescent females [9], do not differentiate between familiar and unfamiliar environments in terms of time spent in social interactions.

These previous experiments, however, assessed age- and sex-related differences in responsiveness to familiarity of the test situation in animals that had been deprived of social contact by housing in isolation for 5–7 days prior to the social interaction test. Most studies also used only an overall measure of social behavior (i.e., overall time spent in social interactions), even though the elementary behavioral acts summed together for these assessments (e.g., sniffing, grooming, chasing, following, crawling under or over, nape attacks, boxing, and wrestling) reflect separable and differentially regulated forms of interactive social behaviors that include social investigation, contact behavior, and play fighting. Each of these forms of social activity has a distinct ethological and ontogenetic pattern [17,20,32,35,39] and is affected differentially by drug treatments [1,29,35]. For instance, the ontogeny of play fighting demonstrates an inverted U-shaped pattern, with a peak being observed around P30–35 [18,19,20,35,39], whereas social investigation is more pronounced in adult than adolescent animals [37]. Taken together, these findings suggest that different forms of social behavior may be mediated by different neural systems, and, hence, may be differentially sensitive across ontogeny to variations in familiarity of the test situation and the amount of social deprivation prior to testing.

Therefore, the main objective of the present study was to determine the impact of social deprivation on social investigation, contact behavior, and play fighting of male and female Sprague–Dawley rats under non-anxiogenic (familiar) or anxiogenic (unfamiliar) test circumstances during ontogeny. The study used a modified social interaction test [39,40] to assess the effects of 5 days of social deprivation or group housing on different components of social behavior and motivation for social contacts in adolescent and adult rats. Given previous reports that environment-dependent changes in social behavior emerge within the adolescent period [27], P28 (early), P35 (mid), and P42 (late) adolescent rats were tested in the study. This work is important, since assessment of social interactions in an anxiogenic situation has been intensively used as an animal model for evaluation of potential anxiolytic drugs in adult animals, whereas information is limited as to the appropriateness of this testing situation for such assessments among adolescent animals (see [8] for references and review).

2. Methods

2.1. Subjects

Sprague–Dawley rats bred and reared in our colony at Binghamton University were used in these experiments. A total of 384 rats derived from 48 litters were used as experimental subjects ($n = 192$, 96 males and 96 females) and partners ($n = 192$, 96 males and 96 females). All animals were housed in a temperature-controlled (22 °C) vivarium maintained on a 14-/10-h light/dark cycle (lights on at 07:00 h) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Pups were housed until weaning with their mothers in standard maternity cages with pine shavings as bedding material. Litters were culled to 10 (5 males and 5 females) pups on P1, the day after birth. Rats were weaned on P21 and placed into standard plastic cages with same-sex littermates (five animals in a cage) until the onset of the differential housing manipulations for this study. In all respects, maintenance and treatment of the animals were in accord with guidelines for animal care established by the National Institutes of Health, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Experimental design

The design of the study was a 4 (age) $\times$ 2 (housing) $\times$ 2 (test situation) $\times$ 2 (sex) factorial, with 6 animals being placed into each of the 32 experimental groups defined by this factorial design. Male and female animals were tested either on P28 (early adolescents), P35 (mid adolescents), P42 (late adolescents), or P70 (young adults). Experimental subjects were housed either in groups of three same sex littermates (group housing) or individually (isolate housing) in standard plastic cages for 5 days prior to the social interaction test. One day before testing (P27, P34, or P41 for adolescents and P69 for adults), experimental animals in the familiar test group were placed into a testing chamber for 30 min to make the testing situation familiar for them, whereas the other experimental animals were placed individually into a holding cage for the same amount of time (unfamiliar test group). To avoid the possible confounding of litter with housing and test situation effects [13], animals were assigned semi-randomly to the experimental groups, with the constraint that no more than one subject of a given sex from a given litter was assigned to a particular housing and test condition. Given that females of different age groups were included in this study, vaginal smears were not used to determine estrus cycle in the adult females.

2.3. Social interaction test

The test apparatuses consisted of Plexiglas (Binghamton Plate Glass, Binghamton, NY) chambers containing clean pine shavings that were proportionally size adjusted to provide comparable social proximity for adolescent and adult animals (30 cm $\times$ 20 cm $\times$ 20 cm for adolescents and 45 cm $\times$ 30 cm $\times$ 20 cm for adults). Each test apparatus was divided along the long axis into two equally sized compartments by a clear Plexiglas partition that contained an aperture (7 cm $\times$ 5 cm for adolescents and 9 cm $\times$ 7 cm for adults) to allow movement of the animals between compartments in a way that only one animal was able to move through the aperture at a time [39,40].

All testing procedures were conducted between 9:00 and 13:00 h under dim light (15–20 lx). Immediately prior to testing, each experimental subject was marked by a vertical line on the back and placed alone in a holding cage for 30 min. This pre-test social deprivation in a novel environment is a standard procedure that has been extensively used to increase baseline levels of social behavior from which inhibitory effects of different experimental manipulations, including anxiogenic compounds, may be more readily detected [6]. Each animal was then placed into the testing chamber simultaneously with a same age and sex test partner. Given that social behavior [22,23,39], social motivation [39] and rewarding

properties of social interactions [4] can be dramatically modified by housing conditions of the partner, partners were always non-manipulated animals that had not been socially isolated prior to testing and who were unfamiliar with both the test apparatus and the experimental animal with which they were paired for testing. Weight differences between test subjects and their partners were minimized as much as possible, with this weight difference not exceeding 5 g for each pair of animals at P28, 10 g at P35, 15 g at P42, and 20 g at P70, and test subjects always being heavier than their partners. During the 10-min test session, the behavior of the animals was recorded by a video camera (Panasonic model AF-X8, Secaucus, NJ), with real time being directly recorded onto the videotape for later scoring (Easy Reader II Recorder; Telcom Research TCG 550, Burlington, Ontario). After each test, the apparatus was wiped with 3% peroxide hydrochloride and the shavings were replaced with fresh ones.

2.4. Behavioral measures

The frequency of a number of social activities [17,32,35,39] of each test subject was analyzed from the video recordings. Social investigation was defined as the sniffing of any part of the body of the partner. Frequency of contact behavior was scored as the sum of crawling over and under the partner and social grooming. Play fighting was analyzed by scoring the frequencies of the following behavioral acts and postures: pouncing or playful nape attack (the experimental subject lunges at the partner with its forepaws extended outward); following and chasing (the experimental animal rapidly pursues the partner); pinning (the experimental subject stands over the exposed ventral area of the partner, pressing it against the floor). Play fighting differs from serious fighting in the laboratory rat by target of attack: during play fighting snout or oral contact is directed toward the partner's nape, while during serious fighting the object of the attack is the partner's rump [24]. In adult animals, serious fighting is characterized by threat postures – a sideways or an upright stance with head and fore body movements toward the partner with attempts to bite (offensive sideways or upright posture) and “serious” attacks – a fierce lunging at the partner's rump often associated with biting [3]. In the present experiments, subjects did not demonstrate threats or serious attacks, and hence frequency of aggressive behavior (serious fighting) was not scored.

Although the main objective of the study was to assess the effect of social deprivation on different forms of social behavior, overall social activity was also scored as the sum of the frequencies of social investigation, contact behavior, and play fighting, therefore providing the possibility to compare the results of the present study with earlier ontogenetic studies, where the overall measure of social activity was the only index used (e.g., [27,28]).

Social preference/avoidance was analyzed by scoring the number of crossovers (movements between compartments) demonstrated by the experimental subject toward the partner and the number of crossovers away from the partner [39,40]. Social motivation was assessed by means of a coefficient of social preference/avoidance [coefficient (%) = (crossovers to – crossovers from)/(crossovers to + crossovers from)]. Social preference was defined by positive values of the coefficient, whereas social avoidance was associated with negative values [39].

In addition to the assessment of social behaviors, total number of crossovers (movements between compartments) demonstrated by each experimental subject was determined and used as an index of general locomotor activity in this social context [37].

Behavioral data were scored from the videotape records by two trained observers without knowledge of the housing and test condition of any animal. Agreement between observers

scoring the same videotaped interactions was in excess of 90% for each measure of social behavior.

2.5. Data analysis

A combined measure of social behavior (overall social activity) and the frequencies of different forms of social activity for each experimental animal were scored. Given experimental findings suggesting that different forms of social behavior may be mediated by different neural systems [1,29,35] and, hence, may be differentially sensitive to social deprivation and variations in familiarity of the test situation, each form of social behavior was analyzed separately. Overall social activity, social investigation, contact behavior, and play fighting, as well as coefficient of social preference/avoidance and overall locomotor activity in the social context (total number of crossovers) were examined in the experimental subjects by using separate 4 (age) $\times$ 2 (housing condition) $\times$ 2 (test situation) $\times$ 2 (sex) analyses of variance (ANOVAs). Where significant interactions involving age, housing condition, and test situation were evident, planned one-way ANOVAs within each test situation/housing condition were performed to further characterize age-related differences. Given that there was no significant main and interaction effects involving sex, this factor was not included in these age analyses. Isolation-induced changes of social behavior under non-anxiogenic (familiar) and anxiogenic (unfamiliar) test circumstances were assessed by post hoc comparisons (Tukey/Kramer test) between isolate- and group-housed animals within each age/test situation condition.

3. Results

3.1. Overall social activity

Analysis of overall social activity revealed significant main effects of age, $F(3, 160) = 75.54, p < 0.0001$, housing, $F(1, 160) = 141.20, p < 0.0001$, and test situation, $F(1, 160) = 188.55, p < 0.0001$, as well as a significant three-way interaction of age, housing, and test situation, $F(3, 160) = 2.74, p < 0.05$ (see Fig. 1). Isolate housing affected overall social activity of adolescent rats, with most pronounced effects seen in early (P28) adolescents. Social deprivation drastically increased interactions with peers at P28 regardless of the test situation, whereas isolate housing was effective in increasing overall social activity only under unfamiliar test circumstances in P35 and P42 adolescents. In contrast, overall levels of social behavior in P70 adults were not affected by social isolation. As seen in Fig. 1, testing in an unfamiliar situation was effective in decreasing overall social activity in isolate- and group-housed animals regardless of age. There were, surprisingly, no effects or interactions involving sex in this analysis or in any of the subsequent analyses that focused on specific components of social behavior, therefore, the factor of sex was not included in further analyses of age-related differences in each housing/test situation condition.

Overall activity of group-housed animals tested in the familiar environment peaked at P35 [main effect of age, $F(3,44) = 7.71, p < .001$], with P35 adolescents differing significantly from all other age groups. In contrast, overall social activity of isolated animals tested either in the familiar or in unfamiliar situation decreased gradually with age [$F(3,44) = 58.97, p < 0.001$ and $F(3,44) = 38.77, p < .001$, respectively], with P28 adolescents showing the highest and P70 adults demonstrating the lowest levels of overall social activity. No age-related differences emerged in group-housed rats tested under unfamiliar circumstances ($p > .05$).

3.2. Social investigation frequency

Social investigation also differed as a function of age, housing, and test situation, $F(3, 160) = 3.91, p < 0.01$ (see Fig. 2). However, in contrast to the overall measure of social behavior,

social investigation was enhanced by isolate housing only in P28 adolescents, an effect seen under both testing circumstances. In contrast, the converse effect was evident in P70 adults, with isolate housing suppressing social investigation of adult animals tested in the familiar environment. Social investigation was suppressed by the unfamiliar environment regardless of age or housing condition. An ontogenetic increase in social investigation was apparent in group-housed animals tested under familiar circumstances, $F(3, 44) = 10.66, p < .001$, with P28 adolescents demonstrating the lowest and P70 animals the highest level of this adult-characteristic form of social behavior. This age-related increase was not evident under unfamiliar test circumstances in group-housed animals ($p > .05$), whereas isolate-housed animals showed similar patterns of age-associated differences in the familiar, $F(3, 44) = 8.11, p < .001$, and unfamiliar, $F(3, 44) = 6.19, p < .01$, test situation, with social investigation of adolescents tested at P28 being significantly more pronounced than of all other age groups.

3.3. Contact behavior frequency

The analysis of contact behavior revealed significant main effects of age, $F(3, 160) = 53.78, p < 0.0001$, housing, $F(1, 160) = 55.47, p < 0.0001$, test situation, $F(1, 160) = 85.93, p < 0.0001$, as well as significant age $\times$ housing, $F(3, 160) = 19.00, p < 0.0001$, and age $\times$ test situation interactions, $F(3, 160) = 3.43, p < 0.05$ (see Fig. 3). Contact behavior was enhanced by isolate housing only in early (P28) and mid (P35) adolescents. Regardless of age or housing condition, animals tested in the unfamiliar environment showed less contact behavior than those tested in the familiar situation. Contact behavior showed an ontogenetic decline under familiar test circumstances in group-housed animals, $F(3, 44) = 10.19, p < .0001$, with the high levels of contact behavior emerging in early and mid adolescents and declining thereafter. Comparable decline in contact behavior from P35 to P70 was observed in isolates tested in the familiar environment, $F(3, 44) = 29.83, p < .0001$, with P28 animals, however, showing extremely higher levels of contact behavior than all other age groups. This behavior also declined gradually with age in isolate-housed animals tested under unfamiliar circumstances, $F(3, 44) = 23.09, p < .001$. In contrast, contact behavior did not differ as a function of age in group-housed animals under unfamiliar, anxiogenic conditions ($p > .05$).

3.4. Play fighting frequency

In general, adolescent animals showed more play fighting than their adult counterparts [a significant main effect of age, $F(3, 160) = 70.31, p < 0.001$], with these age-related differences being further exacerbated by social deprivation [age $\times$ housing interaction, $F(3, 160) = 32.24, p < 0.0001$]. Animals of all ages responded to social deprivation with significant increases in play fighting (see Fig. 4), although these increases were more marked in young adolescents than in older adolescents and adults. Unfamiliarity of test situation significantly reduced play fighting regardless of age or housing [main effect of test situation, $F(1, 160) = 44.12, p < 0.0001$].

Age-related differences in play fighting were more pronounced in animals tested under familiar circumstances [age $\times$ test situation interaction, $F(3, 160) = 3.09, p < 0.05$], with early (P28) and mid (P35) group-housed adolescents showing more play fighting than older (P42) adolescents and adults, $F(3, 44) = 9.39, p < .0001$. Gradual ontogenetic decline in play fighting was evident in isolate-housed rats under familiar, $F(3, 44) = 36.80, p < .0001$, and unfamiliar test circumstances, $F(3, 44) = 28.44, p < .0001$. In contrast to social investigation and contact behavior that did not differ as a function of age in group-housed animals tested under unfamiliar circumstances, ontogenetic differences in play fighting were observed under these housing/test situation conditions, $F(3, 44) = 3.12, p < 0.05$, with P28, P35, and

P42 adolescents demonstrating significantly more play fighting in this anxiogenic test situation than their adult counterparts.

3.5. Social preference/avoidance

In contrast to all other measures of social behavior, social preference/avoidance did not differ as a function of age, and no interactions involving age emerged in the analysis. Group- and isolate-housed animals tested in the familiar environment showed social preference, as indexed by positive values of the coefficient (see Fig. 5), whereas group-housed rats tested under unfamiliar circumstances demonstrated social avoidance (negative values of the coefficient). This social avoidance was eliminated by isolate housing regardless of age and sex [housing $\times$ test situation interaction, $F(1, 160) = 29.36, p < 0.0001$].

3.6. Overall locomotor activity

Analysis of the total number of crossovers between the two compartments of the apparatus, used as an index of overall locomotor activity in the social context, revealed significant main effects of age, $F(3,160) = 22.21, p < 0.0001$ and housing, $F(1, 160) = 194.22, p < 0.001$, as well as a significant age $\times$ housing interaction, $F(3, 160) = 8.60, p < 0.0001$. Overall number of crossovers did not differ as a function of test situation, with animals of all ages demonstrating comparable levels of overall locomotor activity under familiar and unfamiliar test circumstances. Separate for each housing condition ANOVAs revealed that rats of all ages responded to social deprivation with significant increases in overall locomotor activity regardless of test situation (see Fig. 6), although this isolation-induced enhancement of locomotor activity gradually declined with age, $F(3, 92) = 22.32, p < 0.0001$. In contrast, no significant age-related differences in overall locomotor activity emerged among group-housed animals, $F(3, 92) = 1.74, p > 0.05$.

4. Discussion

In the present study, early adolescents were found to be particularly sensitive to the socially enhancing effects of isolate housing. Whereas social deprivation induced increases in all forms of social activity in P28 rats regardless of test situation, older adolescents and adults responded to social deprivation primarily by increases in play fighting. Indeed, when overall social activity was used as a dependent variable, no effects of social deprivation were evident in adult animals under either test circumstance, nor in P35 and P42 adolescents tested in the familiar environment. Thus, when social activity is indexed only as a sum of component social behaviors, this overall measure of social behavior does not appear to be sufficiently sensitive to detect effects of social deprivation across ontogeny.

The activating effects of social deprivation on play fighting were most marked in early adolescents. They demonstrated at least a threefold increase in this adolescent-characteristic form of social interactions under both familiar and unfamiliar test circumstances, with more modest increases in play fighting evident among older adolescents. These results are in agreement with previous reports that play behavior in the rat peaks during early adolescence and declines thereafter [18,19,20,35,39] and that this form of social interactions is extremely sensitive to social deprivation in young animals [12,18,19,31,39]. Reminiscent of our previous findings [39], not only play fighting but also social investigation and contact behavior were elevated in early adolescent animals following social deprivation. Taken together, these results suggest that adolescent animals, especially early adolescents, are extremely sensitive to the activating effects of isolate-housing on social behavior in general and play fighting in particular, with this isolation-induced enhancement of social behavior of early adolescents being evident under both familiar and unfamiliar test circumstances.

Locomotor activity in the social context, indexed in terms of the overall number of crossovers to the partner and from the partner, was also enhanced by isolate housing. However, this isolation-induced stimulation of locomotor activity, in contrast to its social activating effects, was not influenced by age or by test situation familiarity. Isolated animals of all ages demonstrated significantly higher levels of locomotor activity than their group-housed counterparts regardless of test situation, although these locomotor stimulatory effects of isolate-housing were most pronounced in the youngest animals and decreased gradually with age. These results also demonstrated that overall locomotor activity in the social context was unaffected by familiarity/unfamiliarity of the test situation, providing strong evidence that environment-dependent changes in social behavior are unrelated to general locomotor activity [6].

In contrast to overall locomotor activity, all measures of social behavior (overall social activity, social investigation, contact behavior, and play fighting) were sensitive to environmental factors across ontogeny. As expected using this animal model of anxiety [6,8], animals tested in an unfamiliar environment showed less social activity than those tested under familiar circumstances regardless of age, sex, or previous social history. These findings differ from observations of Primus and Kellogg [27,28] who reported that P28 male rats were insensitive to environment-dependent changes in social behavior. The discrepancy between the present study and those of Primus and Kellogg [27,28] may be related to strain differences as well as to substantial differences in pre-test procedures. Sprague–Dawley animals in the familiar experimental condition in the present study were exposed to the test apparatus for 30 min one day prior to testing, whereas Primus and Kellogg familiarized their Long–Evans rats to the test situation for only 7.5 min a day for 2 consecutive days. The short pre-exposure period used in those early studies may have been sufficient for older adolescent (P35 and P42) and adult rats, but perhaps was not long enough to familiarize younger adolescents with the test circumstances, and hence this test context may have remained anxiogenic for the P28 rats. This explanation is supported by the results of the work examining the effects of the anxiolytic drug diazepam in P28 rats tested under familiar and unfamiliar circumstances [28]. In that study, social interactions of P28 rats treated with diazepam were found to differ as a function of test situation, with animals showing more social interactions under familiar than under unfamiliar circumstances—an effect not evident in saline-treated controls. These findings are consistent with the interpretation that, although not as anxious as age-mates completely naïve to the test context, P28 rats pre-exposed to the testing situation were still anxious and displayed moderate levels of anxiety that were effectively counteracted by diazepam.

Although ontogenetic decreases in overall social activity, contact behavior, and play fighting emerged when isolate-housed rats were tested in the unfamiliar environment, these age-related differences in the response to an unfamiliar environment were eliminated by group housing. Reminiscent of previous findings [37], these results demonstrate that the response to an anxiogenic test situation is comparable in group-housed adolescents and adults, even when testing is conducted early in adolescence. The similarity of adolescent and adult responding to the unfamiliarity of the test situation suggests that the social interaction test may be used for assessing effectiveness of different anxiolytic compounds in animals with regular social experience across ontogeny. Indeed, in group-housed animals tested under unfamiliar circumstances, we have demonstrated age-related differences in sensitivity to anxiolytic effects of ethanol, with adolescent animals being less sensitive to ethanol-induced anxiolysis than their adult counterparts [37], and ontogenetic differences in sensitivity to the anti-anxiety properties of ethanol evident even within the adolescent period [38]. With regard to the latter, early adolescents were less sensitive to the anxiolytic effects of ethanol than late adolescents when indexed in terms of increases in social investigation and via the transformation of social avoidance into social preference.

In a conventional social interaction test, a pair of rats is placed into an experimental arena, and in this test situation an animal is unable to avoid social contacts. Therefore, it is difficult to identify and quantitatively characterize the willingness of the tested animal to engage in social interactions (i.e., social motivation). Use of a modified social interaction test [39,40] provides the test subject an opportunity to escape from the partner and allows for the assessment of social motivation, indexed in terms of relative preference or avoidance of the social stimulus. Social preference/avoidance coefficient was the only measure of social activity that did not differ as a function of age in the present study. Group-housed animals of all ages showed social preference when tested in an environment that was familiar to them (but unfamiliar to their partners), whereas similar levels of social avoidance emerged in adolescents and adults when group-housed animals were tested under unfamiliar circumstances. This social avoidance in the unfamiliar test circumstance was transformed into social preference by isolate housing regardless of age. The across-age similarity in isolate-housed experimental subjects was rather surprising, given that all other forms of social behavior were affected differentially across ontogeny, with younger animals being more sensitive to the socially activating effects of social deprivation than their more mature counterparts. Thus, social behavior and motivation for social contacts appear to be differentially sensitive to isolate-housing across age and, hence may have different underlying mechanisms. Moreover, the lack of age-related differences when using the coefficient of social preference/avoidance suggests that this index of social motivation may prove to be a particularly valuable measure for ontogenetic studies of responsiveness to anxiolytic and anxiogenic compounds.

Surprisingly, no sex differences emerged in the present study, with adult females, like their male counterparts, demonstrating more social activity in the familiar than in the unfamiliar test situation regardless of housing conditions. These findings contrast with data from Johnston and File [14] who found that hooded Lister female rats did not increase their social activity when examined in a familiar test situation. Similarly, whereas we observed comparable levels of play fighting in adolescent males and females, some sex differences in components of social interactions have been reported previously among adolescents [21,25,26,32,33], with adolescent males, for instance, engaging in more play fighting [32,33]. Although no main effects or interactions involving sex emerged in overall ANOVAs for each measure of social behavior, social motivation, and locomotor activity, it is possible that the pronounced effects of housing conditions, test situation, and age, as well as interactions of these three factors could have masked possible sex-related differences. Nevertheless, reports of sex-related differences in social behavior appear to be in part strain-dependent. For instance, Long-Evans hooded female adolescent rats engage in less play fighting than their male counterparts, whereas Sprague–Dawley females demonstrate a rate of playful attacks comparable to that of males [21]. Therefore, the lack of sex-related differences in social behavior perhaps should not be surprising, given that Sprague–Dawley rats were used in the present study. The ability of female Sprague–Dawley rats to differentiate between familiar and unfamiliar environments in the present study supports the inclusion of female rats in animal models of anxiety for preclinical psychopharmacological research [2].

In summary, early adolescent animals responded to social deprivation by pronounced increases in all forms of social behavior, whereas depriving older adolescents and adults of social interactions had progressively more modest stimulatory effects, being restricted among adult animals predominantly to play fighting—an adolescent-characteristic form of social interactions. Social investigation – a more adult-typical form of social behavior – was relatively resistant to isolation-induced enhancement and was elevated in early adolescent isolates only. These findings confirm that different forms of social behavior are

differentially sensitive to social deprivation across ontogeny. Neural mechanisms underlying this differential sensitivity to social deprivation remain to be elucidated.

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Fig. 1. Effects of social deprivation on overall social activity of early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).

Fig. 2.

Effects of social deprivation on social investigation of early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).

Fig. 3. Effects of social deprivation on contact behavior of early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).

Fig. 4.

Effects of social deprivation on play fighting of early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).

Fig. 5. Effects of social deprivation on social preference (coefficients > 0) and social avoidance (coefficients < 0) in early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).

Fig. 6. Effects of social deprivation on overall locomotor activity of early (P28), mid (P35) and late (P42) adolescent and adult (P70) rats tested in the familiar and unfamiliar environments. Asterisks (*) indicate significant differences between group- and isolate-housed animals within each age/test situation condition ($p < 0.05$), with data collapsed across sex ($n = 12$ per group).