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Effects of cross-fostering on play and anxiety in juvenile Fischer 344 and Lewis rats

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Abstract

A cross-fostering design was used to assess the relative involvement of genetic variability and early postnatal experiences on differential levels of playfulness in juvenile Fischer 344 (F344) and Lewis (LEW) rats and the extent to which strain differences in anxiety may influence play in these two strains. F344 dams were found to lick and groom their pups less than LEW dams and this was not dependent upon the strain of the pups in the litter. As expected, F344 rats were less playful than LEW rats as demonstrated by fewer playful contacts directed to the nape of a Sprague-Dawley (SD) partner and a decreased likelihood of rotating completely to a supine position when their nape was contacted by the SD partner. These differences could not be readily explained by parallel strain differences in anxiety. The pattern of effects on play as a function of cross-fostering depended on both the genetic background of the pup and the motivational state of the pup prior to testing. Whereas in-fostered LEW pups solicited more play as isolation prior to testing increased from 4 to 24 hours, cross-fostered pups of both strains as well as in-fostered F344 pups were relatively insensitive to the motivational modulation of play solicitation. Responsiveness to play solicitations in pups of both strains reared by F344 dams was insensitive to prior isolation whereas pups reared by LEW dams were less likely to respond with a complete rotation to a supine position when solicited as isolation increased from 4 to 24 hours prior to testing. These data suggest that the overall level of playfulness in a particular strain of rat is fairly resistant to cross-fostering and may be particularly sensitive to genetic variation whereas how this urge is titrated and modified by motivational factors may be influenced more by epigenetic factors.

1. Introduction

Play behavior is commonly observed among the young of many mammalian species and in a variety of other species such as birds, reptiles, and invertebrates [1–3]. Play is believed to have an important role in the developing organism since removing the opportunity to play in young rats has been shown to have a number of adverse consequences on later behavior and social/emotional functioning [4–6] as well as cognitive functioning [7]. In humans, play is almost synonymous with a happy childhood. Conversely, an observed lack of play in an otherwise healthy child can be cause for concern as almost all psychiatric disorders include some type of social dysfunction as a core symptom [8]. This makes the presence or absence

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of play a useful diagnostic indicator when assessing the emotional well-being of a child. Similarly, the relative lack of play in an otherwise playful species could be a sign of some underlying pathology and may be useful for identifying neural substrates associated with psychiatric disorders in which play is disrupted, such as autism, anxiety, and attention deficit/hyperactivity disorder (ADHD).

Rats are a particularly playful species. Rats begin to engage in playful interactions prior to weaning and this continues throughout the juvenile period, peaking at around 35 days of age, and then steadily decreasing as the animals reach puberty [9]. Play in the rat primarily takes the form of “rough-and-tumble” activity; rats will vigorously chase each other, pounce on each other’s dorsal surface, nuzzle the nape, and pin each other [10, 3, 11–13]. Play is quite sensitive to variations in motivational state and is under fairly tight regulatory control. For example, the amount of play observed during a discrete observation period (e.g., 5–15 minutes) can be readily titrated by housing rats in isolation for varied amounts of time [14, 15]; a rat that has been isolated for 4 hours will play more than a rat that has not been isolated, and a rat that has been isolated for 24 hours will play more than the rat isolated for 4 hours. These factors contribute to making the laboratory rat an optimal model for studying the neurobiology of play behavior.

Identifying a strain of rat that plays substantially less than other strains in the absence of any identifiable threat may provide valuable insight into both the genetics and neurobiological substrates that modulate this complex social behavior. For example, the Spontaneously Hypertensive Rat is less playful than either Wistar-Kyoto or Sprague-Dawley (SD) rats [16], Wistar rats are less playful than SD rats [17] and, in our hands, we have identified the inbred Fischer 344 (F344) rat as a strain that is consistently less playful than other strains commonly used in behavioral research [15, 18, 19]. As such, the F344 rat may be a particularly useful model to better understand the extent to which genetic predisposition can impact the neurobiological substrates of playfulness and to gain added insight into those disorders where social dysfunction is a symptom.

In addition to being consistently less playful than other strains, rats of the F344 strain are also hyper-responsive to stress [20–24] and while some studies have reported higher levels of anxiety in F344 rats [25–29] others have reported F344 rats to be less anxious than other strains [30, 31]. In either case, given the extent to which anxiety and stress can negatively impact play in the rat [32] it is possible that play in the F344 rat may be dampened due to altered levels of anxiety in this strain. Since we are unaware of any studies that have assessed anxiety in juvenile F344 rats, anxiety was also assessed in the present study.

Given the magnitude and consistency of the phenotypic differences between F344 and other strains, it is reasonable to speculate that these differences arise largely from genetic variability. If so, then the F344 phenotype should be fairly resistant to modification by early postnatal experiences. However, F344 females have been reported to exhibit less maternal behavior (e.g., less licking and grooming) towards their pups than other strains [33, 34, 19] and it has been known for some time that early interactions between mother and pup can have lasting consequences on the affective lives of rats as juveniles and adults. In particular, rats that receive more licking and grooming as pups tend to be less fearful [35], are more

likely to explore a novel environment [36], and have an attenuated startle response [37] when tested as adults and compared to rats raised by dams that engage in less licking and grooming. Rats that experience brief (e.g., 15 minutes) periods of maternal separation during the first 2 weeks of life are also less anxious and less stressed as adults [38–41].

With regards to play, it has been reported that male pups that receive low levels of licking and grooming tend to be more playful when play is assessed in the home cage and when housed together with male pups that received high levels of licking and grooming and with female pups from litters with high and low levels of licking and grooming [42]. These findings corroborate an earlier study showing that male rats receiving less licking and grooming of the genital area play more than those receiving more licking and grooming [43]. However, these findings are in contrast to two reports showing that male and female pups that experienced brief (e.g., 1–15 minutes) daily periods of separation from the mother play more than those from undisturbed litters [44, 45]. This is particularly intriguing since this procedure of brief daily maternal separation, also known as “handling”, has been reported to increase the amount of licking and grooming by the mother towards her litter [46] and, as mentioned above, leads to the same effects on measures of stress and anxiety as does being raised by a dam on the high end of the licking and grooming spectrum. A recent study looked at play as a function of within-litter variation in maternal care and found a significant positive correlation between the amount of licking and grooming received by male rats during the first week after birth and frequency of pinning, pouncing, and latency to initial social exploration [47]. In other words, male rats receiving more licking and grooming as a newborn were more playful and more socially curious as juveniles. While there may be some inconsistencies with the exact nature of these data it is clear that early postnatal experiences can have an impact on playfulness as a juvenile, most likely through epigenetic modifications [48, 49].

With this in mind, one cannot discount some role for early postnatal experience when accounting for the non-playful phenotype of the F344 strain. In an earlier study [19] we found that cross-fostering F344 rats with Lewis (LEW) dams had no effect on playfulness in that cross-fostered F344 rats played at the same level as in-fostered F344 rats. Since the data from that earlier study was derived from only 2 litters for each fostering condition, the possibility that a “litter effect” [50] could be accounting for these data could not be discounted. Only a cursory assessment of maternal behavior was done in that study as well, making it difficult to draw any conclusions concerning strain differences in maternal behavior. The present study was designed to (1) provide a more robust assessment of cross-fostering on play behavior in F344 and LEW rats, (2) obtain a more detailed assessment of maternal behavior in these 2 strains, and (3) determine whether any of the differences in playfulness may be associated with parallel strain differences in anxiety.

2. Methods

2.1 Subjects and housing

Female F344, LEW and Sprague-Dawley (SD) rats (Harlan, Indianapolis) arrived at 14 days gestation. Rats were housed on corn-cob bedding with ample nesting material in solid-bottom cages (59 cm x 38 cm x 20 cm) with food and water freely available. The colony

room was maintained at 22° C with a 12/12 light/dark cycle (lights on at 08:00). All housing and testing was done in compliance with the NIH Guide for Care and Use of Laboratory Animals using protocols approved by the Institutional Animal Care and Use Committee at Gettysburg College.

2.2 Cross-fostering and maternal observations

All litters were born within 24 hours of each other. F344 and LEW litters were culled to no more than 10 pups and were either cross-fostered or in-fostered within 36 hours of birth. SD litters were also culled to no more than 10 pups and remained undisturbed except for routine cage maintenance until weaning. Beginning on post-natal day 2 (PND2) and continuing for the next 10 days, F344 and LEW litters were observed at various times throughout the day within five possible time windows: 10:00–12:30, 13:00–14:30, 15:00–17:00, 18:00–19:30, 21:00–23:00. Using a protocol originally developed by Myers and colleagues [51] a discrete observation was made every 4 minutes for each F344 and LEW litter over the course of each 1 hour period. With 16 observations in a 1 hour observation period and 48 observation periods over 10 days, this resulted in a total of 768 discrete observations. A total of 39 hours were observed during the light phase and 9 hours during the dark phase. For each observation, the location of the mother (in or out of the nest) was noted. If the mother was in the nest any type of nursing (arch-back, blanket, or passive) was noted along with any licking and grooming of the pups.

2.3 Post-weaning behaviors

Pups were weaned at 21 days of age, weighed, and re-housed in groups of 3–5 in solid-bottom cages (48 cm x 27 cm x 20 cm) with rats of the same sex and same fostering condition. No more than 2 rats of each sex from each litter were used for behavioral testing. Rats were individually handled for 2 days after weaning and were initially tested for play behavior between 28 and 33 days of age. Anxiety was assessed on the elevated plus maze between 36 and 40 days of age and in a novel open field between 46 and 50 days of age. All behavioral testing was done approximately midway through the light phase of the light/dark cycle. Play was assessed in a clear Plexiglas chamber (40 x 40 x 50 cm) that was enclosed within a sound-attenuated wooden chamber illuminated by a single 25W red light bulb. The floor of the testing chamber was covered with approximately 3 cm of Aspen pine shavings.

Play bouts were recorded as digital video files and scored later using behavioral observation software (Noldus Observer XT: Noldus Information Technology) by an observer unaware of the strain of the animal and the fostering condition. All of the rats were initially acclimated to the testing chamber by being placed individually in the testing chamber for 10 minutes on two separate days. Play was assessed over 2 days with each rat tested after 4 hours of isolation and after 24 hours of isolation. At least 48 hours separated the two tests and the order of testing was counterbalanced as much as possible between isolation, strain, sex, and fostering-condition. Each inbred rat was paired with a same-age and same-sex unfamiliar Sprague-Dawley (SD) rat in order to provide a common play partner for each strain. The SD partner was isolated to the same extent as the inbred rat it was paired with and was paired with a different inbred rat for the two tests.

Play was quantified by counting the frequency of contacts directed by the test rat towards the nape of the target SD rat (nape contacts) and the likelihood that a nape contact directed by the target SD rat to the test rat resulted in that rat rotating completely to a supine position (probability of a complete rotation). Nape contacts were quantified by frequency of occurrence while complete rotations were quantified in probabilistic terms by calculating the probability of a complete rotation occurring in response to a nape contact. These two measures of playfulness have been commonly used in this lab and are also thought to be controlled by independent motivational and neural substrates [52, 53, 15, 18, 54]. Rats were re-housed socially after all testing was completed for that day.

Anxiety was assessed by testing rats in an elevated plus maze and by responsiveness to novelty in an open field. Rats were initially tested on an elevated plus maze (Med Associates, St. Albans, VT) using Ethovision video-tracking software (Noldus Information Technology, Netherlands) to track the location of the rat. The elevated plus maze has two open arms (10 cm x 50 cm), two closed arms (10 cm x 50 cm with 40 cm high walls) and a 10 cm x 10 cm center area. Rats were placed in the center of the maze and monitored for 5 minutes. Data analyzed were time in the open arms and total distance traveled.

Open field activity was monitored using a commercially available system (Med Associates; St. Albans, VT) that used infrared (IR) beams to detect locomotor activity in an open field (43.2 x 43.2 x 30.5 cm) placed within a sound-attenuated chamber. Locomotor activity was monitored by 2 sets of 16 beam IR arrays placed 3 cm above the floor of the test chamber and quantified by distance traveled (cm). Anxiety was assessed primarily by time spent in the center of the open field during a 60 minute test session. Using a system-defined zone, a rat was considered in the center when movement occurred further than 6 cm from any wall.

3. Results

3.1 Maternal observations

Observational data from time in the nest, arch-back nursing (ABN), blanket nursing, passive nursing, and licking/grooming (L/G) were each analyzed by a 2 x 2 ANOVA with strain of the pups (F344, LEW) and maternal strain (F344, LEW) as factors (Table 1). Strain of the pups was not significant for any of the observed measures, nor was the pup strain x maternal strain interaction. There was a significant main effect of maternal strain for L/G, $F(1,16) = 7.59$, $p = .014$, with F344 dams engaged in less licking and grooming than LEW dams.

3.2 Body weights at weaning

Upon weaning, an average body weight was calculated for males and females in each litter (Table 2) and these data were analyzed by a 2 x 2 x 2 ANOVA with factors for sex, pup strain, and maternal strain. There was a marginal effect of sex, $F(1,32) = 3.61$, $p = .066$, with females (41.91 ± 0.80) weighing slightly less than males (43.75 ± 0.85). There was a significant effect of pup strain, $F(1,32) = 12.04$, $p = .002$, with F344 pups (41.33 ± 0.79) weighing significantly less than LEW pups (44.33 ± 0.76). There was also a significant effect of maternal strain, $F(1,32) = 12.04$, $p = .002$, with pups raised by an F344 dam (41.15

± 0.87) weighing significantly less than pups raised by a LEW dam (44.51 ± 0.63). None of the interactions were significant.

3.3 Play behavior

Play was assessed by the number of nape contacts directed by the inbred subject towards the SD partner and the likelihood (probability) that a nape contact directed towards the inbred subject by the SD partner resulted in a complete rotation to a supine position. The results for nape contacts can be seen in Figure 1. These data were analyzed by a mixed-factors ANOVA with 2 levels of pup strain (F344, LEW), 2 levels of maternal strain (F344, LEW), 2 levels of sex (male, female), and 2 levels of isolation (4 hours, 24 hours). Pup strain, maternal strain, and sex were between-subjects factors while isolation was a within-subjects factor. Any significant interactions were pursued with separate ANOVAs and t-tests with Bonferroni corrections for multiple comparisons when necessary. As expected, there was a significant main effect of pup strain, $F(1,72) = 29.53$, $p < .001$, with F344 rats directing fewer nape contacts to the SD partner than LEW rats. There was also a significant pup strain x sex interaction, $F(1,72) = 4.51$, $p = .037$. This reflected a sex difference for nape contacts in LEW rats, with males soliciting more play than females, but no sex difference on this measure in F344 rats (Figure 2). There were no other significant main effects or interactions associated with sex for either measure of play. There was a significant pup strain x isolation interaction, $F(1,72) = 8.96$, $p = .004$, as well as a significant pup strain x isolation x maternal strain interaction, $F(1,72) = 8.18$, $p = .006$. To further investigate this interaction, separate ANOVAs with maternal strain and isolation as factors were conducted for F344 rats and for LEW rats. For F344 rats there were no significant main effects or interactions suggesting that neither isolation nor maternal strain affected play solicitation in F344 rats. For LEW rats there was a significant main effect of isolation, $F(1,36) = 7.70$, $p = .009$, with LEW rats soliciting more play after 24 hours of isolation. There was also a significant isolation x maternal strain interaction, $F(1,36) = 4.44$, $p < .042$. A matched-samples t-test with a Bonferroni correction comparing nape contacts after the two isolation conditions found a significant increase in nape contacts after 24 hours of isolation compared to 4 hours of isolation among the in-fostered LEW rats, $t(19) = 3.38$, $p = .003$, but not among the cross-fostered LEW rats.

The likelihood of responding to a nape contact with a complete rotation can be seen in Figure 3. The data were analyzed in the same way as nape contacts. There was a significant main effect of pup strain, $F(1,72) = 10.25$, $p = .002$, with F344 rats less likely to respond to a nape contact with a complete rotation. There was a significant main effect of isolation, $F(1,72) = 7.17$, $p = .009$, as well as an isolation x maternal strain interaction, $F(1,72) = 4.72$, $p = .033$. Further analysis of this interaction indicated that rats reared by a LEW dam were less likely to respond with a complete rotation when isolation increased from 4 to 24 hours, $t(39) = 3.70$, $p = .001$, whereas rats reared by a F344 dam were equally likely to respond to a nape contact with a complete rotation at both 4 and 24 hours of isolation.

3.4 Elevated Plus Maze

For time spent in the open arms there was a significant main effect of pup strain, $F(1,71) = 19.47$, $p < .001$, with F344 rats spending significantly more time on the open arms than

LEW rats (Figure 4). There were no other significant main effects or interactions. There were also no significant main effects or interactions for distance traveled during the 5 minute test (data not shown).

3.5 Activity in a novel open field

For time spent in the center of the open field (Figure 5) there was a significant main effect of pup strain, $F(1,69) = 19.03$, $p < .001$, as well as a significant pup strain x maternal strain interaction, $F(1,69) = 5.31$, $p = .024$, and a significant pup strain x maternal strain x sex interaction, $F(1,69) = 3.99$, $p = .05$. These data indicate that F344 rats were more likely to spend time in the center of the open field and this effect was particularly pronounced in cross-fostered F344 males.

Overall activity in the open field was assessed by distance traveled over the 60 minute test (Figure 6). There was a significant main effect of sex, $F(1,69) = 6.89$, $p = .011$, with females more active than males. There was also a significant main effect associated with maternal strain, $F(1,69) = 4.73$, $p = .033$, with those rats raised by an F344 dam more active than those raised by a LEW mother. No other main effects or interactions were significant.

4. Discussion

Behavioral phenotypes arise from a dynamic interplay between genetic and experiential influences. Play is a fairly stable behavioral phenotype exhibited by the young of many mammalian species and is particularly robust in the juvenile rat. There is also considerable evidence that genetic variability and early postnatal experience can both influence the amount of play exhibited by a rat [55]. We have previously shown that the inbred F344 rat is consistently less playful than other inbred and outbred strains [15, 18, 19] and the present study sought to build on these earlier findings by using a cross-fostering design to begin parsing the relative involvement of genetic variability and early postnatal experiences on the overall playfulness of both F344 and LEW rats. In an earlier study [19] we reported that cross-fostering had no impact on playfulness in the F344 rat. However, given the limited nature of that earlier study, the present work was designed to provide a more robust assessment of cross-fostering on play in F344 and LEW rats. This was done by sampling a smaller number of rats from each litter in order to minimize the risk of any effect, or lack thereof, being due to within-litter variation or “litter effects” [50]. We also sought to obtain a more detailed assessment of maternal behavior in these 2 strains than was done previously and were able to confirm that F344 dams tend to engage in less licking and grooming of their pups than LEW dams. We also examined whether any of the differences in playfulness may be associated with parallel strain differences in anxiety and while we observed strain differences in anxiety, they were not as predicted and could not readily explain differences in play.

Many of the behavioral consequences associated with early postnatal experiences in the rat can often be traced back to the amount of tactile stimulation received through licking and grooming by the mother towards her pups and is thought to operate through epigenetic mechanisms [48, 56, 57]. F344 dams have been reported to engage in lower levels of maternal care than other strains [33, 34, 19] so it is entirely possible that strain differences in

maternal care may be contributing to the playful phenotype of the F344 rat. As expected, F344 dams were found to engage in significantly less licking and grooming than LEW dams while spending a comparable amount of time in the nest and engaged in nursing. No significant differences were found in the amount of licking and grooming between in-fostered and cross-fostered litters for either maternal strain so it can be concluded that F344 rats reared by a LEW dam received more licking and grooming than in-fostered F344 rats. Conversely, LEW rats reared by a F344 dam received less licking and grooming than in-fostered LEW rats. Taken together, these data are consistent with the previous studies mentioned above and suggest that the desired difference in licking and grooming was achieved by cross-fostering in the present design.

Although the mean difference in licking and grooming between F344 and LEW dams was statistically significant, there was still considerable overlap in the amount observed between the two strains. For example, F344 dams were observed to be engaged in licking/grooming during 6.09% of the observation periods with values ranging from 3.44 to 8.50% while LEW dams were observed licking and grooming 8.48% of the observation periods with values ranging from 4.97 to 11.10%. In ongoing experiments we have begun to also monitor maternal behavior in SD dams and, based on preliminary data from 8 litters using the same coding protocol as that used in the present study, licking and grooming has been noted during 10.33% of the observation periods with values ranging from 6.80 to 15.20%. While cognizant of risks associated with comparing data between studies from different labs, it seems noteworthy that the values we are seeing so far with SD dams is fairly consistent with what has been reported by Champagne and colleagues [58] from a much larger sample size. This leaves open the possibility that F344 and LEW dams may both be on the lower end of the distribution for licking and grooming in rats and is worth keeping in mind while interpreting the effects of cross-fostering in the present study.

We have previously reported that separate components of play in F344 and LEW rats may respond differentially to motivational variables [15, 19]. For example, while strain differences in play solicitation (i.e., nape contacts) are more apparent when tested after at least 24 hours of isolation, strain differences in playful responsiveness (i.e., likelihood to respond to a nape contact with a complete rotation) tend to be stable regardless of amount spent in isolation prior to testing. With this in mind, play was assessed after both 4 and 24 hours of isolation. For in-fostered LEW rats, we saw the expected increase in play solicitation (nape contacts) as pre-testing isolation increased from 4 hours to 24 hours. However, there was no comparable difference in play solicitation between 4 and 24 hours of isolation for in-fostered F344 rats, supporting our earlier observations that rats of this strain may be somewhat impervious to the motivational effects of prior isolation on play. When reared by a LEW dam, F344 rats elicited fewer nape contacts than either in-fostered or cross-fostered LEW rats and still failed to show any motivational modulation of play solicitation, suggesting that the lower levels of play solicitation seen in F344 rats are impervious to both rearing condition and pre-testing isolation. Interestingly, nape contacts among cross-fostered LEW rats (i.e., those reared by an F344 dam) also did not differ between the 2 levels of isolation, with the mean number of nape contacts roughly half-way between those seen among in-fostered LEW rats after 4 and 24 hours of isolation. It cannot be determined from these data if this pattern is due to play solicitation in cross-fostered

LEW rats increasing after 4 hours of isolation, decreasing after 24 hours of isolation, or both. So while this measure of play solicitation remains unaffected by cross-fostering in F344 rats, how prior isolation can modulate play solicitation in LEW rats does seem to be impacted if these rats are reared by an F344 dam.

As expected, F344 rats were less likely overall to respond to nape contacts with a complete rotation. It was also found that pups raised by LEW dams were less likely to respond to nape contacts with a complete rotation as isolation increased from 4 to 24 hours and this was found in both LEW and F344 rats. F344 rats reared by LEW dams were still less likely to respond to nape contacts with a complete rotation, but prior isolation affected this measure of play in a manner that resembled in-fostered LEW rats. At the same time, LEW rats reared by F344 dams were still more likely overall to respond to nape contacts with a complete rotation but resembled in-fostered F344 rats in that prior isolation did not affect complete rotations. So while cross-fostering did not modify how prior isolation affected play solicitation (i.e., nape contacts) in F344 rats, it did modify how prior isolation affected playful responsiveness. These data suggest that early postnatal experiences may be particularly influential in modulating how motivational variables impact play in juvenile rats but how this influence is manifested may depend on the genotype that is being acted upon.

Since responding to nape contacts with a complete rotation is thought to prolong a play bout and/or signal playful intent [59] and since our earlier work [19] suggested that playful responsiveness was unaffected by prior isolation, it is unclear why increasing the motivation to play in the present study would lead to a decreased likelihood of a behavioral response thought to prolong play in those pups raised by LEW dams. It is possible that systematic changes in playful solicitations being directed by the SD partner could somehow be affecting responsiveness in some of the fostering conditions. To assess this possibility the nape contact data from the SD rats that were paired with the inbred rats was analyzed in the same way as the inbred data. There were only two significant effects from this analysis. First, there was an expected main effect of isolation, $F(1,72) = 47.3$, $p < .001$, with SD rats exhibiting more nape contacts after 24 hours of isolation (43.8 ± 1.8) than after 4 hours of isolation (29.9 ± 1.3). There was also a main effect of strain of the rat with which they were partnered, $F(1,72) = 11.7$, $p = .001$, with more nape contacts directed to F344 rats (40.9 ± 1.7) than to LEW rats (32.7 ± 1.7). This would also be expected since F344 rats were less likely to direct nape contacts to the SD partner, affording the SD rat more opportunities to direct contacts to the nape of F344 rats. None of the factors involving the strain of the dam were significant suggesting that any quantitative difference in solicitations by the SD partners directed to those rats reared specifically by LEW dams cannot readily explain the drop in complete rotations after 24 hours of isolation. While we don't have an adequate explanation for this isolation-sensitive change in responsiveness it may be that increasing the motivation to play biases behavior towards soliciting play at the cost of responding playfully when solicited, especially within the context of a limited opportunity to engage in play. In either case, while cross-fostering can modulate the extent to which prior isolation affects responsiveness to playful nape contacts overall strain differences on this measure are still relatively unaffected by cross-fostering.

In order to gain additional insight into whether the amount of licking and grooming exhibited by the dam was related to either measure of play, a correlation analysis was conducted between licking and grooming for each litter, nape contacts directed by the inbred rat towards the target SD rat, and the likelihood of the inbred rat responding to nape contacts with a complete rotation. Since 2 rats of each sex were sampled from each litter, an average score was obtained from males and females of each litter for both measures of play after 4 and 24 hours of isolation. There were no significant correlations between amount of licking and grooming and nape contacts for either males or females after either 4 or 24 hours of prior isolation. However, there was a significant negative correlation ($r = -0.53$, $p = .015$) between licking and grooming and the likelihood of a complete rotation in response to a nape contact for males, but only after 24 hours of prior isolation. No other correlations were found to be significant on this measure. This suggests that male rats receiving more licking and grooming are less likely to respond to a nape contact with a complete rotation to a supine position. Male rats receive more licking and grooming from the dam than female rats [34, 47] which may account for why this relationship was only observed in males. As mentioned earlier, rats reared by LEW dams were less likely to respond to nape contacts with a complete rotation after 24 hours of isolation. Since LEW dams exhibited more licking and grooming than F344 dams this could help account for the negative correlation between licking and grooming and playful responsiveness. These data also add support to our overall finding that the impact of motivational state on playful responsiveness is dependent to some extent on the amount of licking and grooming received early in life.

In addition to being consistently less playful than other strains, rats of the F344 strain are hyper-responsive to stress [20–24] and while some studies report the F344 strain to be more anxious than other strains [25–29] other studies have found the F344 strain to be either less anxious [30, 31] or to not differ in anxiety [60, 61]. Given this ambiguity and since these earlier studies assessed anxiety in adults, we considered the possibility that play in the juvenile F344 rat may be dampened due to enhanced anxiety in this strain. Anxiety was assessed in the present study primarily by time spent in the open arms of an elevated plus maze and time exploring the center of an open field. The behavior of F344 rats in both tasks was indicative of lower levels of anxiety in this strain. In particular, F344 rats spent more time in the open arms of the plus maze and more time in the center of an open field than did LEW rats. Given that the two strains differed on both measures of anxiety, albeit in a direction opposite of what was predicted, the possibility remains that some systematic strain differences in the behavior observed on these two tasks may relate to differences in playfulness. While it is hard to imagine increased anxiety being associated with higher levels of play [62], it is possible that strain differences on the elevated plus maze and in the open field may be more reflective of strain differences along some other behavioral dimension, such as responsiveness to novelty. Indeed, we have previously reported that F344 rats spend more time in social investigation when tested with an unresponsive novel rat [19] so it is possible that the increased time in the open arms of the EPM and increased time in the center of the open field may reflect an increased propensity to explore novelty in the juvenile F344 rat.

Although cross-fostering had no impact on the anxiety profile of these strains when tested in the elevated plus maze, cross-fostered male F344 rats that were raised by LEW dams spent

considerably more time exploring the center of a novel open field than any other group suggesting that the propensity of F344 rats to explore when confronted with novelty was amplified in male rats that were reared by LEW dams. Rats that receive more licking and grooming as pups are more likely to explore a novel environment when compared to rats raised by dams that engage in less licking and grooming [36, 63], although these earlier studies only assessed exploration in adult male offspring. Male pups tend to receive more licking and grooming than female pups [34, 64, 47] and when within-litter variation in licking and grooming of male and female pups is taken into account a significant positive correlation was found between the amount of licking and grooming received during the first week and time spent exploring the open arms of an elevated plus maze as an adult for males, but not for females [64]. In other words, male rats that receive more licking and grooming tend to be less anxious as adults. This would be consistent with our finding that male F344 rats raised by LEW dams and, thus, having received more licking and grooming were more likely to explore the center of a novel open field. However, since we did not observe a similar pattern with behavior in the elevated plus maze, these two measures of assessing anxiety may be differentially sensitive to the effects of cross-fostering in these two inbred strains. Given the extent to which behavior in the open field and elevated plus maze may measure different aspects of anxiety [65] it may not be unexpected to find these two measures being differentially sensitive to early postnatal experiences as well. Indeed, this would be similar to an earlier study where it was found that startle response, open field activity, and HPA axis activity were affected differently by cross-fostering in F344 and LEW rats [33]. While the full extent to which cross-fostering may be affecting anxiety in these 2 strains remains an open question that could be more vigorously pursued, it is unclear from these data whether strain differences in anxiety and/or exploration of novelty can readily account for these strain differences in play.

The present data show that the effects of cross-fostering on play in F344 and LEW rats are subtle and dependent on (1) the measure of play that was being assessed, (2), the strain of rat, and (3) the motivational state of the rat as modulated by the amount of isolation prior to testing. For play solicitation, the impact of early postnatal experiences was dependent on the background genotype of the pup in that play solicitation in cross-fostered LEW rats was no longer sensitive to the motivational effects of prior isolation. Unexpectedly, motivation to play also had an impact on how rats responded to nape contacts and this was affected by cross-fostering in both strains. But while cross-fostered LEW rats were insensitive to motivation and resembled in-fostered F344 rats on how prior isolation impacted the likelihood of responding with a complete rotation, cross-fostered F344 rats were now sensitive to motivation and resembled in-fostered LEW rats. It is important to emphasize that these effects of cross-fostering were only apparent in how motivational state affected the relative appearance of the behaviors and not necessarily in the overall amount of the behavior itself for nape contacts or the overall likelihood of that behavior occurring in the case of responding to nape contacts with a complete rotation. This might suggest that the overall impulse to be playful may be particularly sensitive to genetic variation whereas how this urge is titrated by more specific motivational factors such as prior isolation may be influenced more by epigenetic factors.

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Highlights

- A cross-fostering design was used to study strain differences in play
- F344 dams exhibit less licking and grooming of pups than LEW dams
- Cross-fostered F344 rats were still less playful than LEW rats
- Effects of cross-fostering were sensitive to motivational state
- Strain differences in anxiety could not easily account for differences in play

Figure 1.

Mean ($\pm$ SEM) number of nape contacts among in-fostered and cross-fostered F344 and LEW rats tested after either 4 or 24 hours of isolation. The data are collapsed across sex. LEW/LEW = LEW rats reared by LEW dam; F344/F344 = F344 rats reared by F344 dam; LEW/F344 = LEW rats reared by F344 dam; F344/LEW = F344 rats reared by LEW dam. * $p < .05$ when compared to 4 hours isolation

Figure 2. Mean ($\pm$ SEM) number of nape contacts highlighting the sex $\times$ strain interaction. The data are collapsed across rearing condition and isolation. * $p < .05$ when compared to male LEW rats. ** $p < .05$ when comparing F344 to LEW rats

Figure 3. Mean ($\pm$ SEM) probability of responding to a nape contact with a complete rotation among in-fostered and cross-fostered F344 and LEW rats tested after either 4 or 24 hours of isolation. The data are collapsed across sex. Group labels are the same as in Figure 1.

Figure 4.

Mean ($\pm$ SEM) time (seconds) spent in the open arms of an elevated plus maze during a 5 minute test among in-fostered and cross-fostered F344 and LEW rats. Group labels are the same as in Figure 1.

Figure 5. Mean ($\pm$ SEM) time (seconds) spent in the center of a novel open field among in-fostered and cross-fostered F344 and LEW rats during a 60 minute test. Group labels are the same as in Figure 1.

Figure 6. Mean ($\pm$ SEM) distance traveled (cm) in a novel open field among in-fostered and cross-fostered F344 and LEW rats during a 60 minute test. Group labels are the same as in Figure 1.

Table 1

Percentage ($\pm$ SEM) of observation time dams found to be in the nest, engaged in nursing or actively licking/grooming pups for each fostering condition.

Behavior	LEW dam		F344 dam	
	LEW pups	F344 pups	LEW pups	F344 pups
In nest	76.2 $\pm$ 3.4	78.0 $\pm$ 2.9	73.7 $\pm$ 1.9	71.7 $\pm$ 3.1
Arch back nursing	40.9 $\pm$ 5.3	35.0 $\pm$ 3.7	49.3 $\pm$ 6.4	43.0 $\pm$ 7.5
Blanket nursing	19.6 $\pm$ 3.3	20.0 $\pm$ 0.7	15.5 $\pm$ 1.9	16.8 $\pm$ 2.4
Passive nursing	14.4 $\pm$ 4.8	22.4 $\pm$ 2.8	7.9 $\pm$ 4.9	11.1 $\pm$ 5.5
Licking/grooming	8.8 $\pm$ 0.5	8.2 $\pm$ 1.3	6.7 $\pm$ 0.6 *	5.5 $\pm$ 0.8 *

*
p < .05 for main effect of maternal strain

Table 2

Body weights (grams $\pm$ SEM) at weaning. Each data point used to calculate body weight represents an average body weight for each sex in each litter.

Sex	Raised by LEW dam		Raised by F344 dam	
	LEW pups	F344 pups	LEW pups	F344 pups
Male (n = 5/group)	46.46 $\pm$ 1.53	44.40 $\pm$ 0.91	44.23 $\pm$ 1.55	39.86 $\pm$ 1.48
Female (n = 5/group)	44.32 $\pm$ 1.53	43.63 $\pm$ 0.64	42.26 $\pm$ 1.65	38.20 $\pm$ 1.30