

Research report

Sex difference in temporal patterns of social interaction and its dependence upon neonatal novelty exposure

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Abstract

Rodents have been an indispensable tool for the study of the neural mechanisms underlying a variety of emotional, social, and cognitive functions and dysfunctions. Surprisingly, little is known concerning sex difference in rodent social recognition memory and its sensitivity to neonatal stimulation. During the first 3 weeks of life, we exposed male and female neonates to a novel cage for 3-min per day while the matched littermate controls remained in the home cage. At 7 weeks and 7 months of age, we measured frequencies of social investigation over repeated social exposures and found that males showed greater habituation in social investigation than females during both juvenility and adulthood and that neonatal novelty exposure affected changes in the frequency of social investigation across multiple exposures in a sex-dependent manner. We speculate that these observed sex differences may reflect a sex difference in affinity for conspecific novelty rather than memory capability.

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1. Introduction

In social animals, the ability to recognize previously encountered conspecifics is crucial for normal social function. In rodents, social recognition memory can be inferred from a decrease in the frequency or duration of social investigation after repeated exposures to conspecifics [35]. This habituation of social investigatory behaviors towards one animal can be blocked by exposure to a new conspecific—a phenomena referred to as retroactive interference [35]. The ability of the new conspecific to interfere with subsequent habituation to a previously encountered conspecific serves to cross-validate the existence of a memory trace for the previously encountered individual [29].

While there has been a recent increase in social memory research in rodents (e.g. [8,11,12,15,23,29]), very little is known concerning the ontogeny and sex difference in patterns of social interaction after repeated exposures. The majority of rodent social memory studies were conducted in either males or females and at a single point during development. The limited number of studies examining both sexes yielded conflicting results [3,35]. It remains to be determined whether social recognition differs between males and females, how early the onset of this sex difference may be observed, and how this sex difference might change from juvenility to adulthood.

For non-social functions, it is known that early life stimulation represents a major source of variance in sex differences. Neonatal stimulation, with the handling method [9,16], affected the behavior of males and females differently in exploration [41], aggression [34], emotional reactivity [4], and learned helplessness [21]. Parallel to these behavioral differences, neonatal handling also produced sex-dependent

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effects on corticosterone response [22,42], immune response [40], dopamine turnover rate [20], and callosal connections between the two cerebral hemispheres [2]. Neonatal novelty exposure [25] differentially affected male and female rats in open field emotional reactivity [27] and functional brain asymmetry [1].

These early stimulation-induced sex-dependent effects raise the possibility that patterns of temporal changes in the frequency of social interactions induced by repeated social exposures may also be sex-dependent and that sex difference in social interaction may be further modulated by neonatal stimulation. In adult male rats, both short- and long-term habituation to a previously encountered conspecific can be modulated by neonatal novelty exposure [29], a procedure shown to affect a range of functions at multiple levels of analysis [6,7,26,28,30,31,39,45,46]. Here, using a longitudinal design incorporating both male and female rats, we investigated sex-dependent effects of neonatal novelty exposure on changes in the frequency of social investigation across multiple social exposures at juvenility and adulthood.

2. Methods

2.1. Animals

Eight pregnant Long-Evans hooded dams (Charles River, Wilmington, MA) were housed in the Psychology Department vivarium for 16–17 days prior to giving birth. Within 8 h after birth, litters were culled to eight pups with as close to 50% males and females as possible. The number of males ranged from three to seven per litter and the number of females ranged from one to five per litter. A total of 27 male and 29 female rat pups were used for this study. All pups were housed with the dams until weaning at postnatal day 21. After weaning, all animals were housed individually in translucent plastic cages (51 cm × 25 cm × 22 cm) within the same housing rooms and maintained on a 12-h light/dark cycle (lights on at 0700 h) with food and water ad lib. The environment was kept at ambient temperature (21 °C) with humidity at 25%. All procedures were carried out in accordance with the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Neonatal novelty exposure

Neonatal novelty exposure [25] was derived, but differs, from the well-known neonatal handling method [9,16]. On postnatal day 1, approximately one-half of the animals from each litter were pseudo-randomly assigned to the Novel group and the other half to the Home group (split-litter design) with approximately equal numbers of males and females in each group. Group membership was marked via a toe-clipping procedure. On postnatal days 1–21, the dam was first removed from the home cage and placed in a separate cage within the same room (Fig. 1a(i)). The pups were then picked up and identified by the experimenter. Novel rats were placed into separate cages lined with fresh sawdust (ii). After 3 min, Novel pups were returned to the home cage in which Home pups remained (iii). Once all pups were returned, the dam was transferred back into the home cage (iv). During transfer to and from the novel cage, each Novel pup was yoked to a Home pup such that they received a matching amount of experimenter contact at approximately the same time. By

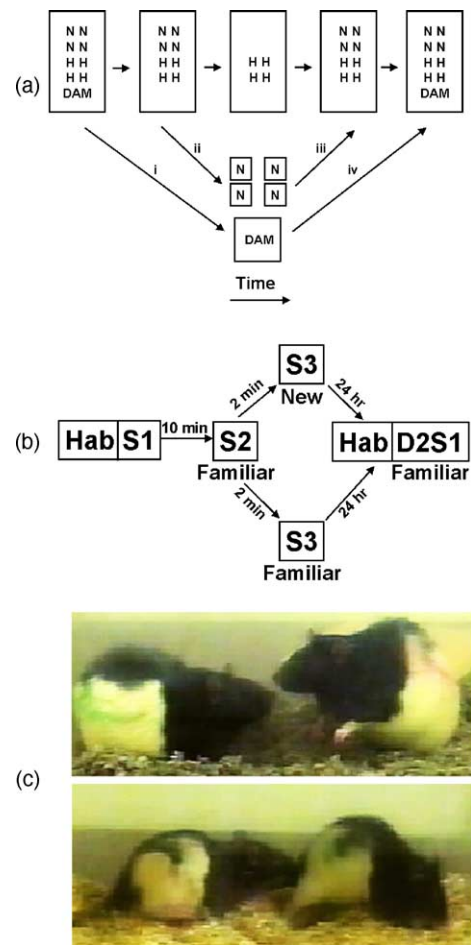


Fig. 1. Methods—(a) the neonatal novelty exposure procedure utilizes a within-litter design and consists of the following: (i) transfer of the Dam to a separate cage, (ii) transfer of the Novel (N) rats to their separate new cages, (iii) return of the Novel rats to the home cage, (iv) return of the Dam to the home cage; (b) the habituation paradigm for social recognition memory; (c) examples of social investigatory behaviors.

using this procedure, any difference in dependent measures between Novel and Home groups cannot be attributed to maternal separation or experimenter contact [25].

2.3. Social recognition memory test

To evaluate the developmental stability of social recognition memory, animals were observed at both 7 weeks and 7 months of age.¹ At each age, the test consisted of two 5-min cage habituation sessions (Hab) and four 5-min social interaction sessions over two consecutive days (Day 1: Hab, S1–S3; Day 2: Hab, D2S1) (Fig. 1b) [for details, see [29]]. During Hab, animals were allowed to habituate to the testing environment. During all other sessions, the pair of rats was allowed to interact with one another. Two pairs of animals were tested simultaneously in cages of the same size and lined with the same bedding as the home cages. Except for S1, which

¹ In this experiment, different stages of the estrus cycle were not controlled for. However, variances in female behavior did not differ from that in males.

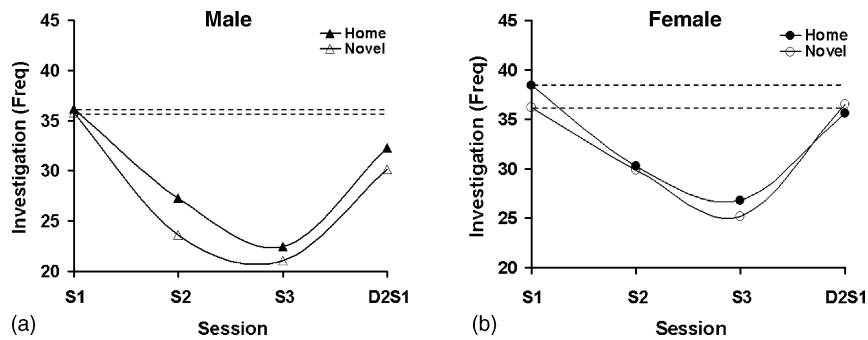


Fig. 2. Effect of neonatal novelty exposure on temporal patterns of social investigation frequency is found only in males (a) but not in females (b).

always immediately followed the Hab session, animals were returned to their home cages during inter-trial intervals (ITIs). ITIs on Day 1 were 10 and 2 min between S1-S2 and S2-S3, respectively. The testing order of male versus female and Novel versus Home groups was counterbalanced. Social investigatory behaviors were videotaped for offline analysis.

The adult-juvenile pairing traditionally used in social recognition memory tests entails a component of social dominance of the testing animal (adult) over the stimulus animal (juvenile). To minimize the probability of aggressive or sexual behaviors, we tested non-sibling, same-sex pairs of similar weight in a neutral testing cage (no aggression was observed in the present study) at both juvenility and adulthood. Similar to the design used in our previous study [29], pairs at 7 weeks of age were formed using two constraints: (1) average within-pair weight difference between Novel and Home rats did not differ significantly from zero, and (2) each animal must have been paired with a non-sibling new conspecific. Given the longitudinal design, it was not possible to satisfy these same constraints at adulthood. Thus, Novel-Home and Home-Home pairing was used at 7 months of age:

Social investigatory behaviors were defined as being proximally oriented to a conspecific (the tip of the nose within approximately 1 cm) or in direct contact while sniffing, following, nosing, grooming, and generally inspecting any body surface [35] (Fig. 1c). The frequency of social investigatory behaviors was measured from sixty 5-s video segments for each of the 5-min sessions. If the behavior was present any time during the 5 s duration, an occurrence of one was counted. Both mutual and uni-directional investigations were included. Short-term habituation (STH) was defined as $(S1-S2)/S1 \times 100$, and long-term habituation (LTH) as $(S1-D2S1)/S1 \times 100$.

During all sessions but Day 1 S3, animals were paired with the same partner (Familiar partner). A subset of animals was exposed to a new conspecific (New partner) on Day 1 S3 (Fig. 1b) for two purposes. The first was to rule out the possibility of generalized social fatigue as a potential confound. A difference in the frequency of social investigatory behaviors during S3 between the Familiar and New partners would serve to rule out social fatigue as a confounding factor [35]. The second was to induce a retroactive interference effect on 24-h habituation. If the animals were able to retain their memory for the new conspecific 24 h later, then we expected to observe a reduction in 24-h habituation (LTH).

2.4. Data analysis

We tested for a litter effect in all dependent measures. If a litter effect was found, we used a general linear model (repeated mea-

asures) with the litter as the unit of analysis and Novelty, Sex, and Age as within factors (SPSS). If a litter effect was not found, individual animals were used as units of analysis. This is clearly indicated by the degrees of freedom reported. Effect size measures of d and f were given for t - and F -tests, respectively [24].²

3. Results

ANOVAs were performed on two types of dependent measures: the frequency of social investigation within one session and habituation of social investigation over two sessions. For frequency of social investigation, repeated measure ANOVA was performed using litter as unit of analysis with Sex, Novelty Exposure, Age, and Session as within-factors. For short-term habituation of social investigation, ANOVA was performed using litter as unit of analysis with Sex, Novelty Exposure, and Age as within-factors. For long-term habituation of social investigation, ANOVA was performed using individual rat as unit of analysis with Sex, Novelty Exposure, and Familiar/New as between-factors. The significant effects from these ANOVAs are detailed below. All other effects were not significant.

3.1. Temporal patterns of social investigation across repeated exposures

ANOVA revealed a significant Novelty by Sex by Session interaction [$F(1, 7) = 10.318$; $p < 0.025$; effect size, $f = 1.214$] (Fig. 2). In males, Novel animals showed a greater rate of habituation within the first three sessions and less recovery after a 24-h inter-trial interval (D2S1) when compared to Home animals (Fig. 2a). In contrast, no such difference was found in females (Fig. 2b). Thus, neonatal novelty exposure affected temporal patterns of social investigation frequency in a sex-dependent manner.

3.2. Average frequency of social investigation

Fig. 3 shows the average frequency of social investigation over all sessions for males and females at two different

² Medium effect size: d , 0.50–0.79; f , 0.25–0.39. Large effect size: $d \geq 0.80$; $f \geq 0.40$.

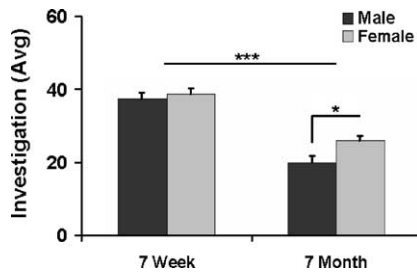


Fig. 3. Sex and Age effects on average frequency of social investigation. * $p < 0.05$, ** $p < 0.025$, *** $p < 0.005$ for all figures.

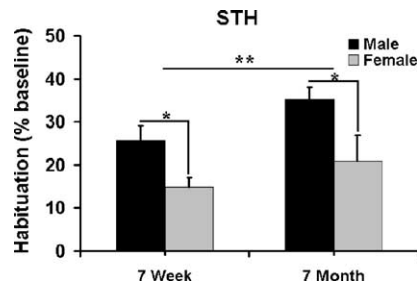


Fig. 4. STH was greater in males than in females at both juvenility and adulthood.

ages. A main effect of Sex was found in social investigation [$F(1, 7) = 12.974$; $p < 0.01$; effect size, $f = 1.361$], with the females showing an overall higher level of social investigation. The main Age effect was also significant [$F(1, 7) = 121.073$; $p < 0.001$; effect size, $f = 4.159$], with juveniles investigating more than adults. Although the Age by Sex interaction was not significant, the overall Sex effect appeared to be mainly driven by a sex difference observed at adulthood ($t(7) = 2.761$; $p < 0.05$; effect size, $d = 2.087$ with Bonferroni adjustment), with adult females investigating significantly more than adult males (Fig. 3, right).

3.3. Short-term habituation of social investigation

A main Sex effect was found in STH scores [$F(1, 7) = 35.819$; $p < 0.005$; effect size, $f = 2.262$], with males showing greater habituation than females at both 7 weeks and 7 months of age (Fig. 4). The main Age effect was also significant [$F(1, 7) = 10.402$; $p < 0.025$; effect size, $f = 1.219$], with adults showing greater habituation than juveniles. The Age by Sex interaction was not significant.

3.4. Retroactive interference on long-term habituation

At juvenility,³ there was no change in the frequency of social investigation between Day 1 S1 and Day 2 S1 ($p > 0.20$), i.e. a lack of 24-h habituation ((S1-D2S1)/S1 $\times 100$). Thus,

³ Due to constraints imposed by weight matching and mortality, it was not possible to have a complete repeated measure design to allow for the testing of Age effect on retroactive interference. Tests were performed in juveniles and adults separately.

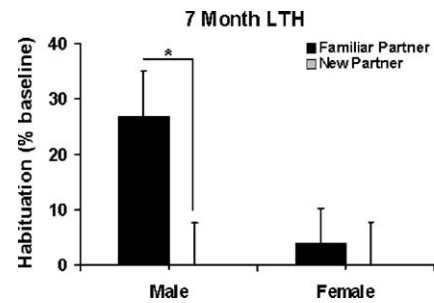


Fig. 5. Retroactive interference effect on LTH was only found in adult male rats.

the following analysis was only performed on adult measures. If there was a retroactive interference effect on LTH, one would expect a difference in LTH between animals that experienced a new or familiar conspecific on Day 1 S3 (Familiar/New). To test for a Sex effect on retroactive interference, ANOVA was performed using Sex, Novelty Exposure, and Familiar/New manipulation as main effects. While the Sex and Novelty effects were not significant, the retroactive interference effect (Familiar/New) was significant [$F(1, 55) = 3.781$; $p < 0.05$; effect size, $f = 0.262$ (one-tailed)], indicating that 24-h habituation can be blocked by a single session of exposure taking place 24 h earlier.

Although the Sex by Familiar/New interaction effect was not statistically significant, the Familiar/New effect on LTH was apparent among only males [$t(25) = 1.902$; $p < 0.05$; effect size, $d = 0.761$ (one-tailed)] (Fig. 5, left). Specifically, male rats who experienced a *new* partner on Day 1 S3 showed no LTH, while male rats that were exposed to the *same* partner throughout all sessions on Day 1 showed LTH that was significantly greater than zero [$t(12) = 2.963$; $p < 0.05$; effect size, $d = 1.711$ (one-sample t -test)] (Fig. 5, left). In contrast, females did not show any signs of LTH ($p > 0.20$) or any difference in their LTH scores between Familiar/New conditions ($p > 0.20$) (Fig. 5, right).

4. Discussion

4.1. Developmentally stable sex difference in short-term habituation

In the majority of rodent studies, social recognition memory was assessed using relatively short delays (≤ 2 h) between the initial and final social exposure. It was generally believed that social recognition memory in rodents lasted less than 2 h when assessed using a habituation paradigm. This presumed short memory duration can be prolonged by social housing [15], neonatal stimulation [29], and pharmacological manipulations [8,11,12,23]. In the present study, we separately measured habituation after a short delay of 10 min and a long delay of 24 h, referred to as short- and long-term habituation, respectively.

While short-term social recognition memory has been studied for the last two decades [35], few studies have investigated the ontogeny of short-term habituation (<2 h) in social investigation. Of these, only males were studied [14,32]. Our study is the first to examine the ontogeny of sex differences in habituation of social investigation from juvenility to adulthood. At 7 weeks of age, males showed significantly greater STH than females. This appears to be the youngest reported age at which sex difference in habituation of social investigation was observed because such a sex difference was absent at 4–5 weeks of age [35]. At 7 months, males habituated more than females, similar to that observed at 7 weeks of age, indicating that sex difference in STH is stable from juvenility to adulthood. Furthermore, we found a general age-related increase in STH from juvenility to adulthood regardless of sex.

It is worthwhile to point out that the direction of sex difference found in this study may be considered contradictory to that found by Bluthé and Dantzer [3], where females showed habituation over longer time delays. However, because their males and females were tested using different testing sequences, it is not possible to determine whether their observed “sex difference” was due to true sex difference or difference in experimental conditions. In contrast, in our experimental design, females and males underwent identical experimental procedures and the order in which they were tested was counterbalanced.

In summary, our findings differ from previous ontogenetic studies of social recognition [14,32,35] in several aspects. First, our study is the first to report an early sex difference in habituation during juvenility. Second, the present study is the first to employ a longitudinal design in which the same animal was measured repeatedly at two points during development. Finally, the pattern of sex difference observed at adulthood replicates that observed at juvenility, thus providing further validation for the present finding.

4.2. Sex differences in interference effect on 24-h habituation

Using a novel experimental design introduced by Tang et al. [29], we further examined sex differences in the sensitivity of long-term habituation (24 h) to retroactive interference effect induced by an exposure to a second (New) conspecific. In that study, the interference effect was used to assess long-term social recognition memory after a 24-h delay. The presence of an interference effect was taken as evidence for the persistence of a memory trace for the new conspecific after a 24-h delay. Here, using the same method, we found that among only adult males, those that were repeatedly exposed to the same conspecific throughout all four sessions, showed 24-h habituation to the first conspecific. In contrast, among those that were exposed to a new conspecific at the end of Day 1 (i.e. on Day 1 S3), 24-h habituation was blocked. This sensitivity of 24-h habituation to a new conspecific indicates that, in males, social memory for the new conspecific can last for

at least 24 h, replicating our previous findings [29]. In adult females, there was no sign of 24-h habituation even among those who were exposed repeatedly to the same conspecific throughout all exposures.

Because a decrease in social investigation after repeated exposure to a conspecific has been considered evidence for social recognition memory, a lack of such habituated social investigation has been typically interpreted as a lack of social memory [35]. However, within the context of sex differences, this interpretation may be challenged. Across many species, females show greater affiliative behaviors than males and such a sex difference is believed to be evolutionarily advantageous for raising young and obtaining protection and food [33]. Perhaps as a consequence of this greater affinity for social interaction, females in several species have been found to be better at recognizing conspecifics [13,37,38]. These cross-species findings challenge the interpretation that the lack of 24-h habituation in the females reported here is a result of failure in social recognition.

It has been hypothesized that males and females differ in their motivation for social interaction [13]. For example, given the option to explore a novel or familiar environment, male and female mice spent 30% and 55% of their time in the familiar environment, respectively [19]. Thus, males appeared to have an affinity for novelty while females preferred familiarity. In social isolation, female rats showed a greater physiological stress response than males, whereas exposure to several conspecifics in a crowded environment reduced stress in females, but increased the stress response in males [5]. Therefore, spatial proximity to conspecifics was aversive to males but calming for females. These sex differences in preference for familiarity and spatial proximity to conspecifics support an alternative interpretation that the smaller short-term habituation and the lack of 24-h habituation in females may be indicative of a motivational rather than a memory difference. Instead of being forgetful, females may simply prefer to spend more time in proximity to a familiar conspecific than males, perhaps due to the different strategies the two sexes employ in maximizing the chance of passing on their genes.

This alternative hypothesis is consistent with our finding that, among adults, female rats displayed a greater overall social interest in same-sex conspecifics than males, as indicated by their higher average frequency of social investigation across multiple social exposures (Fig. 3). Using an intruder paradigm, Thor and Holloway [36] also found greater female–female than male–male social investigation. Using an open field environment, Meaney and Stewart’s [17] study revealed that the combined frequency of sniffing and social grooming was higher in females than in males. Both studies offer additional support for the alternative interpretation that females tend to maintain a higher level of social interaction than males.⁴ More broadly, an extensive body of literature

⁴ One study by Bluthé and Dantzer [3] with a very small sample size (eight males and nine females) and a different definition of social investigation

shows that among both humans and non-human primates, females engage in same-sex affiliative behaviors more than males and that this sex difference may be driven by evolutionary pressure [33].

4.3. Sex differences in sensitivity to neonatal novelty exposure

We found that, in a sex-dependent manner, neonatal novelty exposure affected temporal patterns of changes in the frequency of social interaction across repeated sessions, as indicated by the significant Novelty by Sex by Session interaction effect on the frequency of social investigation. For males, habituation in Novel rats occurred more rapidly within the first three sessions (Day S1–S3) and recovered less after a 24-h delay (D2S1) than in Home rats. In females, similar differences between Novel and Home rats were not observed (Fig. 2). This finding suggests that temporal patterns of social interaction among male rats have a greater sensitivity to early life stimulation than females. This observation is consistent with our previous findings in that neonatal novelty exposure affected males and females differently, in both open field emotional reactivity [27] and functional brain asymmetry [1].

Similar to neonatal novelty exposure, neonatal handling, another early life behavioral manipulation [9,16], also affects male and female animals differentially. For example, only among males but not females did handled rats show greater latent inhibition to shock [43], quicker latency to leave the home cage for exploration [44], and quicker return to baseline measures of corticosterone after delivery of an intense shock [42] than non-handled rats. Neonatal stimulation of the stress response system via corticosterone injection revealed that the frequency of play-fighting behavior was reduced in males but not in females [18]. Along with these previous findings, our result provides converging evidence to support the general conclusion that the effect of early life stimulation differs between males and females.

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