

Neonatal novelty exposure affects sex difference in open field disinhibition

Akaysha C. Tang,^{1,2,3,CA} Masato Nakazawa¹ and Bethany C. Reeb¹

Departments of ¹Psychology, ²Neurosciences and ³Computer Sciences, University of New Mexico, Albuquerque, NM 87131, USA

^{CA,1}Corresponding Author and Address: akaysha@unm.edu

Received 8 April 2003; accepted 28 April 2003

DOI: 10.1097/01.wnr.0000085242.71403.23

Neonatal stimulation induces sexually dimorphic changes at both the levels of behavior and neural systems. The effects of such stimulation on emotional reactivity measured by open field activity have been inconsistent. We found that among 23-day-old rats, neonatal novelty exposure induced an opposite pattern of sex difference in

the initial open field disinhibition. This result suggests that the effect of early life stimulation on emotional reactivity is sex-dependent and that this early stimulation modulates the sexual dimorphism in emotional reactivity to a novel environment. *NeuroReport* 14:1553–1556 © 2003 Lippincott Williams & Wilkins.

Keywords: Emotional reactivity; Neonatal; Novelty; Open field; Rat; Sex difference

INTRODUCTION

The open field has been used to evaluate an animal's emotional reactivity to a relatively unfamiliar environment [1,6]. Emotional reactivity is typically indexed by an animal's level of activity within single or multiple sessions of open field exposure. The greater the amount of activity an animal exhibits, the less emotionally reactive the animal is considered. When the open field exposure begins with placing the animal in the center of the field, rats tend to show a freeze response and leave the center with slow and tentative movement, i.e. a low level of activity [7]. The open field activity appears to be inhibited by the novel testing environment. If the animal is repeatedly exposed to the same open field there can be an increase in activity between the first two trials of exposure as a result of disinhibition.

Neonatal handling, an early life stimulation method [6,8], has been repeatedly demonstrated to reduce emotional reactivity in the open field [6]. Recognizing that the neonatal handling procedure involves three components (handling of the pups by the experimenter, separation from the dam, and exposure to an unfamiliar environment) we recently developed a new procedure called neonatal novelty exposure which isolates the last of these three components [7]. As a result of this procedural modification, we were able to conclude that repeated brief exposures to a novel cage during the first three weeks of life was sufficient to induce a reduction in emotional reactivity, whereas separation from the dam and the experimenter handling were not necessary for inducing a reduction in emotional reactivity in the open field. This procedure has also been shown sufficient to result in enhanced learning in the Morris water task [7], enhanced long-term retention of an odor-reward association [7], enhanced social recognition memory [9], enhanced modula-

tion of synaptic transmission by the stress hormone corticosterone [10] and enhanced synaptic plasticity [11,12].

Because these previous neonatal novelty studies focused on male rats, it is not known whether neonatal novelty exposure will affect the females similarly. Neonatal handling has been shown to have sex-dependent effects on the following dependent measures: exploration [13], tail pinch-induced asymmetry [4], immobility in the swim test [14], immune response [15], stress response [14], brain monoamines [16] and callosal area [2]. However, the results from open field studies are inconsistent with regard to sex-specific handling effects [3,5,15,17,18]. Most of the studies did not find a sex by handling interaction effect.

Although these previous studies primarily focused on whether the effects of neonatal stimulation differ between males and females, the same data sets could also be used to examine whether neonatal stimulation potentiates or diminishes sex difference. In other words, one could examine whether the development of sex differences in these behavioral and neural measures can be facilitated by neonatal stimulation. In the present study, we measured the initial disinhibition, a dynamic aspect of open field behavior, among both male and female rats that experienced either neonatal novelty exposure or the matched control condition. We found that neonatal novelty exposure significantly modified patterns of sex differences in disinhibition to a novel open field.

MATERIALS AND METHODS

Animals: Six pregnant Long-Evans hooded dams arrived at the Psychology Department vivarium 14 days prior to giving birth (Charles River, Wilmington, MA). The litter size

of each dam varied from 9 to 16 pups. Of the pups born, a total of 22 male and 29 female rats were used in this study. The dams and pups were housed in translucent plastic cages ($51 \times 25 \times 22$ cm) with a 07.00-19.00 h light:dark cycle with food and water *ad lib*. Pups were housed with their dams until weaning at postnatal day 21. After weaning, all animals were housed individually.

Neonatal novelty exposure: Within 8 h after birth, litters were culled to eight pups with ~4 males and 4 females in each litter. Half of the males and half of the females in each litter were randomly assigned to the Novel group while the other half were assigned to the home group (split-litter design). Group membership was distinguished by marking both hind paws. Each day, from postnatal day 1 to 21, the dam was first transferred to and remained in a separate cage. The novel pups were then picked up and placed in a novel non-home cage lined with fresh sawdust for 3 min while the home pups stayed in the home cage. During transfer to and from the novel cage, each home pup was yoked to a novel pup such that every time a novel pup was touched by the experimenter, its yoked home pup was also similarly touched. This insures that both novel and home groups were separated from their dam for an equal amount of time and that they received the same amount of experimenter handling. Thus, the only treatment difference was the brief exposures to the non-home cage. For more details of this procedure see [7].

Open field test: At 23 days of age and two days after weaning, animals were exposed to an open field ($60 \times 60 \times 20$ cm) during four 20 s trials. An experimenter who was blind to the treatment condition tested animals in groups of eight. Each trial in the present study began with placing the animal in the center of the open field (to maximize the initial fear response). At the beginning of each trial, the animal was briefly covered by a cardboard box of similar size to the animal's body length and width. When the box was lifted, the animal was allowed to freely ambulate. After each trial, the animal was returned to its home cage, which was placed within the testing room. In order to minimize interference with the animal's behavior, the experimenter remained at the same location in the room during all trials. Open field sessions were videotaped by a camera mounted directly above the open field for offline analysis. Activity levels were defined as the number of squares traversed and were measured offline by a coder who was blind to treatment conditions.

Open field testing parameters differ widely from study to study. A single session could be as long as several minutes [5,22] or as short as 20 s [7] as was in the present study. The number of sessions can vary from one [5,17] to over a dozen [3]. The time span for multiple sessions ranges from <1 h (present study) to >2 weeks [3]. We reasoned that the longer two rats are exposed to an identical environment during a session, the more similar their behavior will become. Averaging or totaling the activity levels over a longer period of time in the open field might have made the initial differences in the open field behavior undetectable in some of the previous studies. Thus, we used a short trial duration (20 s per trial) to increase the likelihood of

capturing individual differences in the initial responses upon first entering a novel environment. Furthermore, we used short intertrial intervals (ITIs; 5 min) to more rapidly reduce the initial fear response to the novel environment over repeated trials. This combination of 20 s trial duration and 5 min ITIs has been previously demonstrated to be effective in revealing a neonatal novelty exposure effect on the open field activity in male rats [7].

Statistical analysis: Because multiple rats from the same litter were used, we first tested for a litter effect on these dependent measures. Significant litter effects were found which suggested the need for using the litter, instead of individual rat, as the unit of analysis. Consequently, novelty exposure, sex, and trial were considered as within-factors. Based on previous studies, we predicted specific patterns of results. Accordingly, contrasts and directional tests were used.

RESULTS

Emotional reactivity is typically measured by average activity across several trials [6]. Our previous study suggests that testing for a group difference using an average measure of several trials may fail to detect group differences present in the dynamic aspects of trial-to-trial behavior. Specifically, in a water maze study [7], the novel and home groups did not differ when their performance was indexed by an average swimming latency measure, yet novel animals displayed more rapid reduction in swim latency across the initial two trials, thus showing more rapid learning.

Here, to capture the dynamic aspect of open field emotional reactivity, we similarly examined changes in open field activity across the initial two trials (T1 and T2). Rats typically became briefly immobilized upon entering the center of an open field for the first time [7]. Based on this previous finding, we expected to find an initial increase in open field activity from T1 to T2. Such an increase in activity can be considered to reflect how quickly an animal becomes disinhibited to an initially novel environment. For the purpose of comparison, we also performed statistical tests on the more conventional average measure as well as a trial one (T1) baseline measure. All analyses were conducted using litter as the unit of analysis.

Figure 1a shows that there was an initial decrease between T1 and T2 in the length of time it took the rats to become ambulatory and to leave the center square. This latency remained stable between T2 and T4. Figure 1b shows a corresponding increase between T1 and T2 in the amount of open field activity and a later small decrease between T3 and T4. Both open field activity level and the latency to leave the center square showed a significant quadratic trend (activity: $F(1,5)=30.531$, $p=0.003$; latency: $F(1,5)=17.751$, $p=0.008$).

Trial-to-trial open field activity for the novel males and females is shown in Fig. 2a and for the home males and females in Fig. 2b. The baseline emotional reactivity was not significant for neither the main effects nor the sex by novelty interaction ($p>0.20$). The interaction effect on the average emotional reactivity measure was nearly significant ($p=0.074$). In contrast, the novelty by sex interaction effect on the initial disinhibition measure (T2–T1) was significant

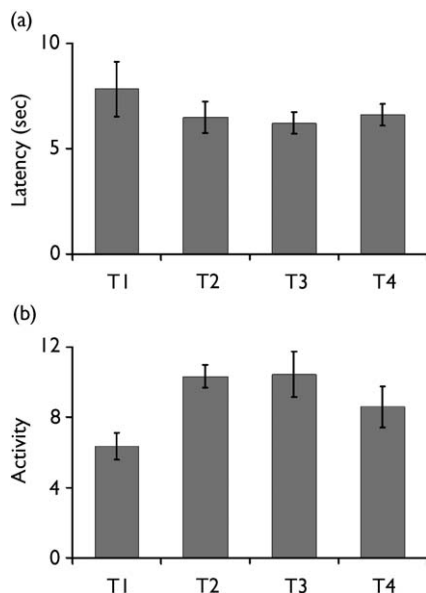


Fig. 1. Temporal patterns of open field behavior across four consecutive trials (T1–T4) for all animals. (a) latency (time to move away from the center square) and (b) activity (number of squares traversed).

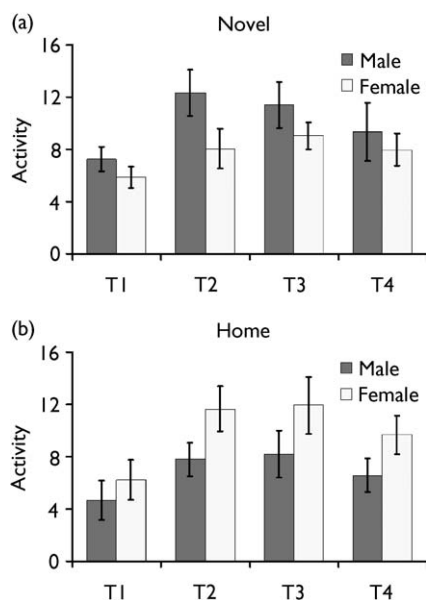


Fig. 2. Temporal patterns of open field activity across four consecutive trials (T1–T4). (a) Novel males versus novel females. (b) Home males versus home females.

($F(1,5)=9.072$, $p=0.015$; effect size $\eta=0.803$; Fig. 3c). Among the novel animals, the disinhibition was higher for the males than for the females (Fig. 3, right). An opposite pattern was found among the home rats, with the disinhibition in the females higher than in the males (Fig. 3, left). Similar analysis performed on the latency to leave the center square did not yield any statistically significant results although similar patterns were found.

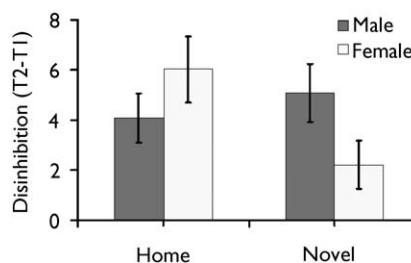


Fig. 3. Neonatal novelty exposure potentiates sex differences in the initial increase of open field activity from T1 to T2.

DISCUSSION

Modulation of sexual dimorphism in open field disinhibition by neonatal novelty exposure: In the open field, rats are known to show emotional reactivity, indicated by a lack of movement when initially exposed to a novel environment [7] and to show a disinhibition, or a reduction in this emotional reactivity, by an increase in locomotion as they become familiarized to the initially novel environment [6]. In contrast to the typical measure of average activity levels, we used the changes in activity between the first two exposures to measure behavioral disinhibition in a novel open field. We were able to demonstrate a significant sex by neonatal novelty interaction effect on this initial disinhibition in the open field (Fig. 3). This sex difference is opposite in direction for the novel and home animals: among the novel animals, males showed a greater initial disinhibition than females; among the home animals, females showed a greater initial disinhibition than males. This pattern of sex differences indicates that neonatal novelty exposure modulates sexual dimorphism in the dynamic adaptation to a novel environment.

Possible mediating mechanisms: Neonatal stimulation via handling [6,8] and neonatal novelty exposure [7] has been shown to produce a cascade of physiological [19,20], neurophysiological [10–12] and neuroanatomical [20,21] changes. Among these changes, the most reliable finding is that neonatal stimulation results in a reduction in the duration of the hypothalamic-pituitary-adrenal (HPA) axis response to stress [19,20]. Not only does neonatal stimulation produce parallel changes in HPA-axis and open field behavior at the level of treatment groups, it has also recently been shown that corticosterone concentration measured after behavioral stress correlates significantly, at the level of individuals, with open field activity (unpublished data). Interestingly, the difference in stress responses between males and females is similar to that between the handled and non-handled. The adult males have a shorter lasting corticosterone response than the females to physiological stress [22] and a smaller corticosterone response to exposure to a novel environment [13,23]. These similar patterns suggest that HPA-axis is a potential converging point at which early life stimulation exerts its modulatory influence on sexual dimorphism.

Origin of increased sex differences in open field disinhibition: How neonatal novelty exposure facilitates sex differ-

ences in open field disinhibition is not yet clear. The interaction between maternal behavior and neonatal novelty exposure may be one source of this facilitation. Dams are known to display a discriminative response towards her pups, with greater licking and grooming directed towards males than females [24]. Neonatal handling increases licking and grooming in general [25], and thereby can amplify any existing preferential maternal care towards the male. Because dams showing greater licking and grooming have offspring with reduced corticosterone responses to acute stress [25], this amplified sex difference in maternal care is likely to result in an amplification of sex difference in stress response. Therefore, neonatal novelty exposure can enhance sex difference in stress response via existing maternal discrimination. The enhanced sex difference in stress response in turn can manifest itself behaviorally as an enhanced sex difference in disinhibition to novelty presented in an open field among the neonatal novelty exposed animals relative to their matched controls.

Significance of increased sex differences in open field disinhibition: The observation that a very subtle neonatal stimulation can produce, among 23-day-old animals, a sex difference in disinhibition to a novel environment opposite to that among the controls has important implications. First, sexual dimorphism in a non-sexually oriented behavior can be induced quite early by a uniformly applied early life experience. Second, because sex differences can be observed very early on in life, observation based on one sex alone should not be casually generalized to the other sex, as is often done implicitly in many biomedical studies. Third, because the observed sex by neonatal novelty interaction is manifested in behavioral response patterns to novelty, such an interaction may also affect measures of cognitive functions under novel learning or testing situations.

Relationship to previous studies: Several studies [5,15,18] failed to detect an interaction between sex and the handling treatment. This discrepancy between previous studies and the present results may be due to differences in trial duration (3–20 min *vs* 20 s) and inter-trial intervals (24 h *vs* 5 min). By spreading testing trials over a longer time period than that used in the present study, Bronstein *et al.* [3] detected a sex by handling interaction with the direction of the interaction opposite to changes observed in the present study. The main cause of the difference between these two studies is most likely that the present study dealt with short-term dynamic changes in response to a novel environment

while the Bronstein *et al.* study dealt with long-term changes. It is not presently known whether neonatal novelty exposure will affect short- and long-term response changes to the open field differentially.

CONCLUSIONS

Using the initial increase in activity as a measure for disinhibition and a novel open field testing sequence, consisting of four 20 s trials with ~5 min ITIs, we observed, among 23-day-old animals, that neonatal novelty exposure induced an opposite pattern of sex difference in this open field disinhibition. This finding suggests that sex differences in emotional reactivity to a novel environment can be modulated at a very early age by neonatal stimulation. Whether neonatal stimulation can modulate sex differences in related cognitive functions as well as in synaptic plasticity remains to be explored.

REFERENCES

1. Archer J. *Anim Behav* **21**, 205–235 (1973).
2. Berrebi A, Fitch R, Ralphe D *et al.* *Brain Res* **438**, 216–224 (1988).
3. Bronstein P, Wolkoff F and Levine M. *Behav Biol* **13**, 133–138 (1975).
4. Camp D, Robinson T and Becker J. *Physiol Behav* **33**, 433–439 (1984).
5. Cannizzaro C, Martire M, Cannizzaro E *et al.* *Brain Res* **904**, 225–233 (2001).
6. Denenberg V. *Psychol Rev* **71**, 335–351 (1964).
7. Tang A. *Learn Mem* **8**, 257–264 (2001).
8. Levine S. *Science* **126**, 405 (1957).
9. Tang AC, Reeb BC, Romeo RD and McEwan BS. *J Neurosci* in press.
10. Zou B, Golarai G, Connor JA and Tang A. *Dev Brain Res* **130**, 1–7 (2001).
11. Tang A and Zou B. *Hippocampus* **12**, 398–404 (2002).
12. Zou B, Reeb B and Tang A. *Soc Neurosci Abstr* **648**, 17 (2002).
13. Weinberg J, Krahn E and Levine S. *Dev Psychobiol* **11**, 251–259 (1978).
14. Papaioannou A, Gerozissis K, Prokopiou A *et al.* *Behav Brain Res* **129**, 131–139 (2002).
15. von Hoersten S, Dimitrijevic M, Markovic B and Jankovic B. *Physiol Behav* **54**, 931–940 (1993).
16. Papaioannou A, Dafni U, Alikaridis F *et al.* *Neuroscience* **114**, 195–206 (2002).
17. Padoin M, Cadore L, Gomes C *et al.* *Behav Neurosci* **115**, 1332–1340 (2001).
18. Weizman R, Lehmann J, Leschiner S *et al.* *Pharmacol Biochem Behav* **64**, 725–729 (1999).
19. Levine S. *Sci Am* **202**, 80–86 (1960).
20. Meaney M, Aiken D, Bhatnager S *et al.* *Science* **239**, 766–769 (1988).
21. Verstynen T, Tierney R, Urbanski T and Tang A. *Neuroreport* **12**, 3019–3022 (2001).
22. Kitay J. *Endocrinology* **68**, 818–824 (1961).
23. Wiener S and Levine S. *Physiol Behav* **31**, 285–291 (1983).
24. Moore C and Morelli G. *J Comp Physiol Psychol* **93**, 677–684 (1979).
25. Liu D, Diorio J, Tannenbaum B *et al.* *Science* **277**, 1659–1662 (1997).