Behavioural Brain Research 224 (2011) 318–325

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Dissociation between neonatal novelty-induced preferential maternal care and enhancement in cognitive, social, and emotional functions

Bethany C. Reeb-Sutherland[a,1], Akaysha C. Tang[a,b,*]

[a] Department of Psychology, University of New Mexico, Albuquerque, NM 87131, United States
[b] Department of Neurosciences, University of New Mexico, Albuquerque, NM 87131, United States

ARTICLE INFO

Article history:
Received 2 March 2011
Received in revised form 6 June 2011
Accepted 9 June 2011
Available online 17 June 2011

Keywords:
Maternal care
Maternal mediation
Maternal modulation
Post-natal handling
Neonatal handling
Neonatal novelty exposure
Spatial memory
Social memory
Aggression
Behavioral inhibition

ABSTRACT

Early life stimulation is known to produce long-lasting changes in the brain and behavior. One such early stimulation method is the neonatal novelty exposure procedure which allows the isolation of the novelty effect from several prominent confounding factors inherent to the neonatal handling procedure. In two previous studies, we found long-lasting novelty effects on different sets of functional measures without accompanying preferential maternal care, even when the observation was made immediately after the novelty manipulation, a time when such preferential care is most likely to be expressed. Here, within a single cohort of Long-Evans male rats, we demonstrate that novelty exposure leads to enhancements across several functional domains, including increased disinhibition to novelty, enhanced spatial and social memory, and reduced aggression, again without the accompaniment of preferential maternal care. These findings extend novelty exposure effects to aggression and replicate previously known novelty exposure effects on spatial and social memory with extension to new developmental stages. Most importantly, these findings do not support the hypothesis that preferential maternal care towards novelty-exposed pups mediates the observed novelty effects. We discuss the possibility that the effects of neonatal novelty exposure are *mediated* via repeated activation of the hypothalamic–pituitary–adrenal (HPA) axis that serves to inoculate pups for future exposures to novelty and novelty-induced HPA activation and that maternal influence is likely to be expressed via its *modulatory* role—the mother sets the individual-family specific behavioral and hormonal context to allow the same early life experience to have a family-specific effect.

1. Introduction

In rodent studies of early experience on development, the neonatal handling procedure has been one of the most frequently utilized manipulations. Over half a century ago, when Levine and colleagues [1] first set out to investigate the effects of neonatal stress induced by repeated electric shocks delivered to rat pups, they made an unexpected discovery – both the repeated-shock (Shock) and control (referred to as Handling) groups produced similar effects on the offspring's stress response, a faster initiation and recovery of the corticosterone stress response, in comparison to a third group that received only the standard laboratory rearing treatment (referred to as Non-Handling) [1]. Since this paradoxical finding, neonatal handling has become a frequently used paradigm for studying early experience effects on adult function [2,3]. In comparison to the Non-Handling treatment, Handling treatment involves at least the following additional factors: pup stress due to novelty exposure, separation from the dams, and handling by the experimenter and maternal stress due to her separation from the pups [4,5]. Therefore, handling as a name for the Handling treatment is more of a misnomer that can easily lead to casual unsupported conclusions, when, in reality, the actual observations only support the conclusion that it is at least some combination of the above that led to these changes in offspring physiology.

To address these well-known methodological problems and consequently improve interpretability of findings, the neonatal novelty exposure procedure was introduced to isolate the novelty component from the other confounding factors that are inherent to the neonatal handling procedure. Other laboratories have since replicated the effectiveness of this procedure in creating novelty-induced changes in the brain and behavior [6,7]. By utilizing a within-litter design, this procedure entails exposing half of a litter to a relatively novel non-home environment (Novel) while keeping the other half within the familiar home cage (Home). Therefore,

* Corresponding author at: University of New Mexico Albuquerque, NM 87131, United States. Tel.: +1 505 277 4025; fax: +1 505 277 1394.
E-mail address: akaysha@unm.edu (A.C. Tang).
URL: http://atlab.unm.edu (A.C. Tang).
[1] Present address: Department of Human Development, University of Maryland, College Park, MD 20742, United States.

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.06.010

the novelty effect is determined by comparing two groups of sibling pups that share the same mother. By further matching the experimenter handling and maternal separation between the Novel and Home siblings within the same litter, this procedure allows one to draw conclusions regarding the novelty exposure effect without differences in experimenter handling, pup separation stress, and maternal separation stress between Novel and Home pups that confound the effects of novelty. At the level of behavior, this neonatal novelty exposure procedure has previously been shown to lead to enhanced spatial memory [7–9], improved social recognition memory [10], increased recovery from novelty-induced behavioral inhibition [6,11], and increased competitive success in winning limited resources [12].

For several decades, it has been suggested that maternal care mediates the effects of early stimulation [13–18, but see also 19,20,21] based on findings of correlated handling-induced changes in the offspring and handling-induced changes in maternal care. In the case of novelty exposure-induced changes, although the novelty-exposed and home-staying siblings share the same mother, it cannot be presumed that the mother treated them similarly upon their reunion, particularly given that dams are known to discriminate between her disturbed pups, that were taken away from the nest to experience a low-temperature environment, and undisturbed pups, who stayed in the nest [22]. Therefore, some may suggest that the various effects of neonatal novelty exposure may be similarly correlated with preferential maternal care directed towards novelty-exposed rats, analogous to the greater amount of maternal care displayed by dams of the pups that experienced the handling treatment. However, it has been shown that despite a lack of retrieval-based preferential maternal care, a measure observed immediately after the novelty exposure treatment and shown to be positively associated with subsequent active nursing of the pups, novelty-induced enhancement in adult offspring's spatial memory was nevertheless observed [23]. Furthermore, in a separate study, novelty-induced enhancement in social competitiveness and the plasticity of the hypothalamic–pituitary–adrenal (HPA) axis was accompanied by a lack of retrieval-based preferential maternal care [12,23]. Together, these findings do not appear to support the hypothesis that preferential maternal care mediates novelty-induced enhancements in spatial memory, social competitiveness, and plasticity of the HPA axis.

Here, we aim to investigate, in a single cohort of rats, whether effects of neonatal novelty exposure across multiple behavioral endpoints—previously examined either separately or without a simultaneous examination of possible preferential maternal care—can be demonstrated with or without preferential maternal care. Secondly, we sought to investigate whether this dissociation can be consistently observed, because in two previous studies, one found preferential care towards the home-staying instead of the novelty-exposed pups [23] and the other found a lack of such preference [12]. A final goal of the current study was to extend the set of novelty exposure effects to include previously unexplored functions, specifically social aggression, and to extend known novelty effects to ages previously not examined.

2. Materials and methods

2.1. Experimental animals

All experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with approval by the Institutional Animal Care and Use Committee at the University of New Mexico. Twenty-two pregnant Long-Evans hooded dams (Harlan, Indianapolis, IN) arrived at the Psychology Department vivarium 10–12 days prior to giving birth. Upon arrival, each dam was individually housed in a translucent plastic cage (51 cm × 25 cm × 22 cm) and maintained on a 12-h light/dark cycle (lights on at 8:00 a.m.) with food and water *ad libitum*. Temperature and humidity were 21 °C and 25% respectively. The litter size ranged between 6 and 14 pups at the time of birth and was culled within 8 h of birth to 8 pups per litter to maintain similar litter size across dams. During culling, if the number of male pups were fewer than 8, female pups were kept to maintain the 8-pup litter size. Pups were housed with the dams until weaning on postnatal day (PND) 21 from which time on the pups were housed separately in translucent plastic cages (51 cm × 25 cm × 22 cm) without further environmental enrichment. Of the pups born, only the male offspring (N = 106) were used in this study.

2.2. Neonatal novelty exposure

On PND21, approximately one-half of each litter was pseudo-randomly assigned to the Novel and the other half to the Home conditions (split-litter design), with body size counter-balanced between the two groups by visual inspection. Apparent body size rather than precisely measured body weight was used to minimize experimenter manipulation that was not pertinent to key experimental manipulations. In comparison to the default random assignment procedure, by taking apparent body size into consideration, additional information was used to counter-balance the distribution of body size across the Novel and Home groups, thus contributing to an improved experimental design. Group membership was marked by tattooing the left and right hind paws of the Novel and Home pups using two different digit combinations: (1) left first and right fifth, and (2) right first and left fifth. The precise patterns of marking were counterbalanced between the Novel and Home groups across litters.

During PND1–21 between 10:00 and 15:00 h, the neonatal novelty exposure procedure (Fig. 1A and B) was carried out in the animal housing room and involved half of each litter spending 3 min away from the familiarity of their home cage (Novel group) and their matched control siblings remaining in the home cage (Home group). Specifically, the dam was first removed from the home cage. The Novel and Home pups were then identified by examining toe markings. Once identified, Novel rats were placed in a new cage (30 cm × 19 cm × 13 cm) lined with fresh bedding for their 3-min exposure and subsequently returned to their home cage in which the Home rats remained. During this transfer, each Novel pup was yoked to a Home pup that received a matching amount of experimenter contact at approximately the same time as the yoked Novel pup. The dam was returned to the litter after both the Novel and Home pups were reunited in the home cage. The amount of touching by the experimenter and the duration of maternal separation during this novelty exposure procedure was matched between the Novel and Home rats, thus ensuring that any difference in outcome measures between the two groups was attributable to neither dam separation nor experimenter touch. It should be noted that the total duration of separation between dams and all of her pups was less than 15 min, which does not constitute the kind of prolonged maternal separation (>3 h) known to result in deficits in offspring emotional, cognitive, and neuroendocrine function [for review, see 24].

This procedure is in contrast to the commonly used neonatal handling procedure [1–3] that utilizes a between-litter design in which the Non-Handled litters remained entirely undisturbed and the Handled litters experienced a combination of at least four manipulations: (1) "handling" of experimental animals by the experimenter; (2) separating the neonates from their mother; (3) increasing the mother's stress by separating her from her pups; and (4) exposing the neonates to an unfamiliar environment, i.e. novelty. Therefore, by using a split-litter or within-litter design, the novelty exposure component is isolated from the other three confounding factors, including maternal stress and associated maternal behavioral differences, inherent to the neonatal handling procedure [8].

2.3. Offspring behavioral measures

Offspring reactivity to a novel environment, spatial working memory, social recognition memory, and aggression were investigated longitudinally in the same cohort of rats (Fig. 1a). These functions have been previously examined at ages different from those in the present study or within the context of other experimental manipulations.

2.3.1. Disinhibition to novelty (open field test)

When exposed to a novel environment, animals typically show at least a brief period of behavioral inhibition, expressed as freezing or displaying little movement, followed by a period of increased exploration, which we refer to here as disinhibition. This rapid change in the initial behavioral response to novelty was examined using a unique open field procedure consisting of multiple shortly spaced 20-s long exposures [11,25]. In contrast to the typically used open field testing parameters which include several minutes (up to 30 min) of continuous exposure, this procedure allows efficient assessment of the rat's initial response to a novel environment.

Specifically, on PND24, animals were exposed to a novel open field (60 cm × 60 cm × 20 cm) during eight 20-s trials. An experimenter who was blind to the treatment condition tested animals in groups of eight. To maximize the initial fear of novelty, individual pups were placed in the center of the open field. At the beginning of each trial, rats were briefly covered by a cardboard box similar to the size of their body. The trial began immediately after the box was lifted and the rat was allowed to ambulate freely. To minimize interference with the rat's ongoing behavior, the experimenter remained still and in the same location during all trials. All trials were videotaped by a camera mounted directly above the open field. Activity level was defined as the number of 12 cm × 12 cm squares traversed. To quantify