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Neural and environmental factors impacting maternal behavior differences in high- versus low-novelty-seeking rats

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ABSTRACT

Selective breeding of rats exhibiting differences in novelty-induced locomotion revealed that this trait predicts several differences in emotional behavior. Bred High Responders (bHRs) show exaggerated novelty-induced locomotion, aggression, and psychostimulant self-administration, compared to bred Low Responders (bLRs), which are inhibited and prone to anxiety- and depression-like behavior. Our breeding studies highlight the heritability of the bHR/bLR phenotypes, although environmental factors like maternal care also shape some aspects of these traits. We previously reported that HR vs. LR mothers act differently, but it was unclear whether their behaviors were genetically driven or influenced by their pups. The present study (a) used cross-fostering to evaluate whether the bHR/bLR maternal styles are inherent to mothers and/or are modulated by pups; and (b) assessed oxytocin and oxytocin receptor mRNA expression to examine possible underpinnings of bHR/bLR maternal differences. While bHR dams exhibited less maternal behavior than bLRs during the dark/active phase, they were very attentive to pups during the light phase, spending greater time passive nursing and in contact with pups compared to bLRs. Cross-fostering only subtly changed bHR and bLR dams' behavior, suggesting that their distinct maternal styles are largely inherent to the mothers. We also found elevated oxytocin mRNA levels in the supraoptic nucleus of the hypothalamus in bHR versus bLR dams, which may play some role in driving their behavior differences. Overall these studies shed light on the interplay between the genetics of mothers and infants in driving differences in maternal style.

Introduction

Rats, like all organisms, display a wide variety of behavioral and physiological responses when placed in a new or stressful situation. Such individual differences in environmental or emotional reactivity in humans influence personality and temperament, and may ultimately place certain people at risk for developing stress-induced pathology, including depression, anxiety and a host other psychiatric and addictive disorders (Ball et al., 2005). We have developed selectively-bred rat lines which capture two extremes of environmental or emotional reactivity (Stead et al., 2006a). When placed in a novel environment, selectively-bred High Responder (bHR) rats exhibit a high level of exploratory activity, while bred Low Responder (bLR) animals are very inhibited, showing much less exploration. Several studies show that this single behavioral trait (high- versus low-novelty exploration) strongly predicts a number of behavioral features, including anxiety (Kabbaj et al., 2000; Mallo et al., 2007; Stead et al., 2006a; White et al., 2007), depression (Mallo et al., 2007; Orr et al., 2008), aggression (Abraham et al., 2006), impulsivity (Flagel et al., 2010), and drug-taking behavior (Davis et al., 2008; Hooks et al., 1991; Piazza et al., 1989, 1991a). We have generally observed that bLR rats exhibit a highly anxious/depressive-like syndrome and are sensitive to the negative effects of chronic stress (e.g. prenatal stress (Clinton et al., 2008) and chronic mild stress (Stedenfeld et al., 2009)), while bHRs are prone to be aggressive, impulsive, and self-administer drugs of abuse.

Numerous studies have identified a series of neurobiological factors that may underlie the marked bHR/bLR phenotypic differences (Ballaz et al., 2007; Cecchi et al., 2007; Hooks et al., 1994a,b; Kabbaj, 2004; Kabbaj et al., 2000; Piazza et al., 1991b; Rosario and Abercrombie, 1999). For instance, differences in drug- and reward-seeking in commercially available (i.e. non-selectively-bred) HR/LR rats are likely associated with their distinct dopaminergic circuits (Hooks et al., 1994a,b; Lucas et al., 1998; Piazza et al., 1991b), although such differences may also stem from their distinct hypothalamic–pituitary–adrenal (HPA) axis reactivity (Maccari et al., 1991; Piazza et al., 1991a; Rouge-Pont et al., 1998). Other work using Affymetrix

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microarrays identified numerous putative gene expression differences in the hippocampus of commercially purchased HR/LR rats both basally and following psychosocial stress, highlighting differences in a range of molecules involved in intracellular signal transduction pathways, neuroplasticity, and neurogenesis (Kabbaj et al., 2004). In addition, our selective breeding of the bHR/bLR lines has shown the high level of heritability of the bHR/bLR phenotypes, with the traits being highly predictable based on parental phenotype (Stead et al., 2006a). Thus, there appears to be a strong genetic component that underlies the emergence of the bHR and bLR phenotypes.

A vast literature illustrates the profound impact of early life experience on emotional and neuroendocrine stress reactivity (Arnold and Siviy, 2002; Denenberg et al., 1967; Hofer, 1973; Ladd et al., 2000; Levine, 1962; Levine et al., 1957, 1967; Russell, 1973; Sanchez et al., 2001; Zarrow et al., 1972). The first 3 postnatal weeks represent a critical developmental period for rodent neural systems, evident in radical changes in behavior, neuroendocrine response, synaptic connectivity, and global neural gene expression (Altman and Sudarshan, 1975; Card et al., 2005; Eilam and Golani, 1988; Rinaman et al., 2000; Sapolsky and Meaney, 1986; Smart and Dobbing, 1972; Stead et al., 2006b; Wiedenmayer and Barr, 1998). From P4–P14, rodents exhibit a 'stress hypo-responsive period' (SHRP) characterized by a reduced capacity to secrete corticosterone in response to stressful stimuli, which protects the developing brain against the deleterious effects of elevated glucocorticoids. Maternal behavior is critical for maintaining the SHRP period as well as a variety of other physiological processes in developing infant rats (Levine, 2001). Thus, it is perhaps not surprising that stress-induced perturbations of mother–pup interactions, or even naturally occurring variation in mothering-styles, can markedly impact neurodevelopment and subsequent behavior (Fleming et al., 1999; Francis et al., 1996; Meaney and Szyf, 2005). Given the broad behavioral and neuroendocrine differences between bHR/bLR (as well as commercially available HR/LR) animals, we have been interested in studying whether these distinct phenotypes are shaped by early life experience, specifically in terms of the quality and/or quantity of maternal care that they receive in the first postnatal weeks. We recently demonstrated that commercially purchased HR–LR dams display marked differences in maternal styles during the first two postpartum weeks, with LR dams spending more time licking, grooming, and arched-back nursing their pups compared to HR dams (Clinton et al., 2007). In the present study, we hypothesized 1) that the selectively-bred bHR and bLR dams would exhibit similar maternal behavior differences; 2) that bHR and bLR dams would show neurobiological differences that may contribute to their maternal behavior differences; and 3) that cross-fostering studies would reveal whether the bHR/bLR maternal styles are inherent to the mothers themselves, or driven by the phenotype of the pups that they are raising.

Materials and methods

Animals

bHR and bLR animals were acquired from our in-house breeding colony where we have maintained the bHR/bLR lines for many generations. We recently published a description of our breeding strategy and initial behavioral characterization of the bHR and bLR lines (Stead et al., 2006a). Rats were housed in 43×21.5×25.5-cm polycarbonate cages (Nalgene, 24×45×20) throughout the studies. The rooms were kept under constant temperature (25±2 °C) and lighting conditions. Mating pairs as well as dams and litters during lactation were housed on a 14:10 light–dark cycle (lights on at 6:00 a.m.). Food and tap water were available *ad libitum*. All experiments were conducted in accordance with the National Institute of Health (NIH) guidelines on laboratory animal use and care, dictated by the National Research Council in 1996, and all procedures were approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC).

Mating bHR–bLR animals

bHR and bLR females were paired for 14 days with bHR and bLR males, respectively. Mating pairs were kept on the 14:10 light–dark cycle as this has been shown to promote regular estrous cycles and fertility (Everett and Sawyer, 1949; Ying et al., 1973). Conception was verified by the presence of a vaginal plug. Pregnant females were individually housed on the calculated eighteenth day of gestation, and litters were culled to 12 healthy pups (6 males, 6 females) on the day of birth (P0). After the initial handling of cages at birth, the mothers and litters were not disturbed in order to minimize disruption of mother–pup interactions, except for weekly cage change. Pups were weaned on postnatal day 21 and grouped 4 animals per cage according to sex, with water and food available *ad libitum*.

Monitoring maternal behavior in bHR/bLR dams

bHR dams and litters ($N=12$) and bLR dams and litters ($N=12$) from the 14th generation of our colony were observed from postnatal day 1 to 14 using a protocol similar to that used by Myers et al. (1989). Each cage was observed twice daily — once during the light phase (at approximately 2:00 p.m.) and once during the dark phase (at approximately 10:00 p.m.). Each observation period (lasting 45 min to 1 h) consisted of a series of 10 5-sec "snapshot" observations for each cage, which were taken approximately 5 min apart. During a "snapshot" observation, a checklist was used to note which behaviors were being observed. The behaviors noted were: 1) mother in or out of nest; 2) mother in contact with any pups; 3) mother in contact with more than half of the litter; 4) mother licking or grooming a pup; 5) mother transporting a pup; 6) mother manipulating non-nest bedding; 7) mother manipulating nest bedding; 8) mother eating; 9) mother drinking; 10) mother self-grooming; 11) mother rearing; 12) mother resting away from litter; 13) mother passive nursing pups; 14) mother arched-back nursing pups. Passive nursing was defined as the mother lying on her side or back and nursing any number of pups. Arched-back nursing was classified as the mother arched over any number of nursing pups with her legs extended. By the end of the 14 observation days, each cage had accumulated 280 observations (10 observations per session×2 sessions each day×14 total days).

In situ hybridization studies in brains of bHR and bLR dams

bHR and bLR dams ($N=8$ per group) from the 12th generation of our selectively-bred lines were sacrificed by rapid decapitation between 8:00 AM and 10:00 AM on postpartum day 8, a timepoint when we previously observed marked maternal behavior differences in commercially purchased HR/LR dams (Clinton et al., 2007). Brains were removed, snap frozen, stored at −80 °C, and later cryostat sectioned at 12 μm. Sections were taken at 240 μm intervals from the tip of the frontal cortex through the midbrain, and then prepared for *in situ* hybridization as previously described (Clinton et al., 2008). Briefly, sections were fixed in 4% paraformaldehyde at room temperature for 1 h. The slides were then washed three times in room temperature 2× SSC (300 mM NaCl/30 mM sodium citrate, pH 7.2), 5 min each wash. Next, the slides were placed in a solution containing acetic anhydride (0.25%) in triethanolamine (0.1 M), pH 8.0, for 10 min at room temperature, rinsed in distilled water, and dehydrated through graded ethanol washes (50%, 75%, 85%, 95%, and 100%). After air-drying, the sections were hybridized with a ^{35}S-labeled cRNA probe for oxytocin or the oxytocin receptor. The probes were labeled in a reaction mixture consisting of 1 μg of linearized plasmid, 1× transcription buffer (Epicenter Technologies, Madison,