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Variations in maternal behavior in rats selected for infant ultrasonic vocalization in isolation

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ABSTRACT

Individual differences in maternal behavior in rodents are associated with altered physiology and behavior in offspring across their lifespan and across generations. Offspring of rat dams that engage in high frequencies of high-arched-back nursing and pup-licking (High-LG) show attenuated stress responses compared to those engaging in lower frequencies (Low-LG). Selective breeding also produces widespread alterations in physiology and behavior that are stable over generations. To examine processes underlying generational and developmental influences on anxiety in an animal model, we developed two lines of rats that emit either extremely high (High-USV) or low (Low-USV) rates of 45 kHz ultrasonic vocalizations in isolation at postnatal day 10. Compared to the Low-USV line, High-USV rats display increased indices of anxiety- and depression-like behavior in adulthood. The current study assessed maternal behaviors as well as oxytocin and vasopressin receptor density in High-USV and Low-USV dams to determine if selective breeding had produced differences that paralleled those found in Low- and High-LG dams. We found that Low-USV dams engage in more high-arched nursing and pup-licking than High-USV dams. Differences in oxytocin and vasopressin receptor levels were not widespread throughout the brain, with line differences in the piriform cortex and nucleus accumbens. This research illustrates the potential interplay between genetically determined (USV line) and environmental (postnatal mother–infant interactions) factors in accounting for the phenotypes associated with maternal separation induced postnatal vocalizations.

Introduction

In the first research program to selectively breed for a mammalian infantile trait, we developed two lines of N:NIH rats that, as postnatal day (PND) 10 infants in isolation, emit either extremely high (High-USV line) or very low (Low-USV line) rates of 45 kHz ultrasonic vocalizations (Brunelli et al., 1997). The breeding design, created to model aspects of human separation anxiety, also produced alterations in the lines' physiology and behavior across development from infancy through adulthood. Compared with randomly-bred N:NIH rats, both High- and Low-USV juveniles show reduced frequency and quality of play, as well as reduced 50 kHz USV during play (Brunelli et al., 2006). High-USV adults characteristically show more anxiety-like (open field) and depressive-like (Porsolt swim) behaviors than Low-USV adults (Brunelli and Hofer, 2007). At PND18, High-USV juveniles and adults exhibit sympathetic over-activity regulating heart rate, whereas Low-USV juveniles and adults show parasympathetic under-activation of heart rate (Brunelli and Hofer, 2007; Brunelli et al., 2002). In adulthood, Low-USV males are also more aggressive in male–male social interactions (Brunelli and Hofer, 2007). Components of female sexual behavior are reduced in the Low versus the High-USV lines, as are levels of midbrain and plasma 3α, 5α-THP (allopregnanolone), which are known to regulate sexual behavior (Frye et al., 2006; Zimmerberg et al., 2005). These phenotypic changes, based on selection for an early infant isolation-induced behavior are stable across generations (Brunelli and Hofer, 2007).

Naturally occurring individual differences in the maternal behavior of rats are also associated with lifelong variation in behavioral and neuroendocrine responses to stress and novelty in their offspring. Many, but not all, of these differences parallel those observed between the High- and Low-USV lines. Adult offspring of rat dams exhibiting high rates of maternal licking (anogenital and non-anogenital) and high-arched nursing (High-LG dams) show attenuated corticosterone responses to stress and reduced anxiety-like behavior compared to adult offspring of dams whose rates of high-arched nursing and licking are significantly lower (Low-LG dams) (Caldji et al., 1998; Cameron et al.,

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2005; Champagne et al., 2003; Francis et al., 1999). Similar to the Low-USV line, female offspring of High-LG dams show reduced sexual receptivity (Cameron et al., 2008). Play behavior is reduced in both male and female juvenile High-LG compared to Low-LG offspring, suggesting some distinction from the USV lines, where play is reduced in both Low- and High-USV individuals (Brunelli et al., 2006; Parent and Meaney, 2008). Differences in maternal behavior between High- and Low-LG dams are stable and the impact on offspring persists across generations (Francis et al., 1999).

Thus, variation in sexual behavior, anxiety, stress responses, behavioral or physiologic traits, and other phenotypes can be linked to either selective breeding for infant USV rates or to variation in the quality of early mother–infant experiences. In light of parallel effects observed in the phenotypes of High- and Low-USV lines and High- and Low-LG offspring, here we address the question of whether there are also parallel differences in the early maternal environment of the USV lines. Early in the selection process, we found that cross-fostering of pups at PND1 between High- and Low-USV line dams did not produce significantly different USV rates at PND10 compared to in-fostered pups of the same line, indicating that there was no postnatal maternal contribution to the selected behavior, USV rate (Brunelli et al., 2001). Nonetheless, the question arises as to whether maternal behaviors in the first week of lactation exist that might account for other differences between lines that emerge later in life. We hypothesized that Low-USV dams would engage in elevated levels of arched-nursing and pup-licking and thus would be comparable to High-LG dams. Investigation of the neurobiological basis of the Low- versus High-LG maternal phenotype has implicated region-specific changes in the density of oxytocin receptors in hypothalamic and limbic structures. Though the related vasopressin system has not been explored in Low- versus High-LG dams, recent evidence has demonstrated that vasopressin does promote specific forms of maternal care in rats (Bosch and Neumann, 2012). Accordingly, here we hypothesized that the presence of group differences in maternal behavior in High- and Low-USV lines would be complemented by variation in oxytocin and vasopressin receptor density in the maternal brain.

Materials and methods

Subjects and housing

All procedures used in this study were reviewed and approved by the Columbia University and New York State Psychiatric Institute's Institutional Animal Care and Use Committee. Originally, 25 breeding pairs of N:NIH rats, specifically developed for selective breeding, were provided by the NIH (Hansen and Spuhler, 1984). The phenotype selected is based on the number of USVs emitted by postnatal 10-day-old (±1) pups in 2 min of isolation. Over many generations, all pups from all litters born were screened for isolation-induced USV rates at PND10 (±1). In the High- and Low-USV lines respectively, individual pups vocalizing for the highest and lowest rates of vocalization within litters were selected as breeders for the next generation. The lines were maintained separately as closed breeding systems, and mating occurred only within lines. Breeders were mated with partners from litters outside the natal litter, and mating pairs did not share parents or grandparents in common (Brunelli et al., 2001). Subjects in the current study were PND90–120 females from High- and Low-USV line litters representing the 30th to 35th selected generations. Vocalization rates in these generations for the High-USV line averaged 162 calls/min (N = 250, range: 110–200), while Low-USV line call rates averaged 18 calls/min (N = 348, range: 10–23). The USV rates of adult subjects in this study were not assessed during infancy, nor were their litters. Subjects' litters were sacrificed on PND6; this obviated assessment of pup rates at the standard postnatal age of 10 days. However, litters of 9 High-USV and 8 Low-USV dams' half-siblings were tested, and were comparable to the dams themselves. A two-sample *T*-test indicated significant differences between means of two separate distributions [$t(128) = 25.12$, $p < 0.001$]. A Kolmogorov–Smirnov Two-Sample Test ($p < 0.001$), confirmed that these two samples did not come from the same distribution. 12 High-USV and 13 Low-USV line females were bred with their respective line males between 90 and 120 days of age. At approximately gestational day 18, pregnant females were singly-housed in clear, polypropylene cages with pine bedding. Room temperature and humidity were regulated, a reversed light cycle was implemented (lights off: 0800; lights on: 1800), and food and water were available ad libitum. The day of birth was designated PND0 and litters were culled to 8–10 pups per litter, balanced by sex when possible.

Maternal behavior

Observations of maternal behavior were based on a procedure developed by Myers et al. (Myers et al., 1989). The occurrence of the following behaviors was assessed: *high-arched nursing* — dam is in a high-arched posture over pups with legs splayed; *low-arched nursing* — dam is over the litter, but is not arched and with no extension of her legs; *passive nursing* — dam is lying on her side with one or more pups attached; *licking or grooming* — dam licking the pup (either anogenital or body); *nest-building* — dam moving or carrying pine shaving bedding. A total of 12 High- and 12 Low-USV dams were observed for maternal behavior, each for five 75-minute observation periods daily for the first 6 days postpartum. Multiple observers were trained to a high level of inter-rater reliability (>0.90). Within each 75-minute observation period, the behavior of each mother was scored every 3 min (25 observations/period × 5 periods per day = 125 observations/mother per day). Frequency of a behavior was calculated as the number of times the behavior was observed divided by the total number of observations. Total contact time with pups and a composite measure of high-arched nursing/licking and grooming were also calculated based on observed behavioral frequencies.

Oxytocin and vasopressin receptor autoradiography

Immediately following the last observation on day PND6, dams were sacrificed through rapid decapitation and brains were removed and placed briefly in liquid nitrogen, then stored at −80 °C. Brains were sectioned in the coronal plane at 20 μm, and sections thaw-mounted onto poly-L-lysine coated slides. Slide-mounted coronal brain sections were processed for autoradiography using ^{125}I d(CH2)5[Tyr-Me)2,Tyr-NH29] oxytocin receptor (New England Nuclear, Boston, MA) and vasopressin V1a receptor autoradiography using ^{125}I-lin-vasopressin [125Iphenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH2] (New England Nuclear, Boston, MA) as previously described (Champagne et al., 2001; Curley et al., 2012). All autoradiograms were analyzed using an image analysis system (MC1D-4, Interfocus Imaging, Cambridge, UK). Between three and six sections were analyzed bilaterally for each brain region. Oxytocin receptor binding was analyzed in the following regions (with respect to Bregma): (1) Dorsal lateral septum, ventral lateral septum, nucleus accumbens core, and nucleus accumbens shell (+2.52 mm to −1.92 mm), (2) Bed nucleus of the stria terminalis and posterior ventral lateral septum (+0.60 mm to −0.48 mm), (3) Medial preoptic area and ventromedial preoptic nucleus (+0.24 mm to −0.48 mm) and (4) Arcuate nucleus, central amygdala, cortical amygdala, posterior dorsal medial amygdala, intermediodorsal thalamic nucleus, posterior ventral medial amygdala, and ventromedial hypothalamus (−2.04 mm to −3.24 mm) (Paxinos and Watson, 2005). Vasopressin V1a receptor binding was analyzed in the following regions (with respect to Bregma): (1) Dorsal lateral septum, nucleus accumbens core, nucleus accumbens shell, and ventral lateral septum (+2.28 mm to +0.84 mm), (2) Interstitial nucleus of the posterior limb of the anterior commissure, lateral preoptic area, medial preoptic area, piriform cortex, septohippocampal nucleus, and septohypothalamic nucleus (+0.48 mm to −0.48 mm) and (3) Central amygdala, hippocampus,