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Variation in maternal and anxiety-like behavior associated with discrete patterns of oxytocin and vasopressin 1a receptor density in the lateral septum

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ABSTRACT

The relationship between anxiety and maternal behavior has been explored across species using a variety of approaches, yet there is no clear consensus on the nature or direction of this relationship. In the current study, we have assessed stable individual differences in anxiety-like behavior in a large cohort (n = 57) of female F2 hybrid mice. Using open-field behavior as a continuous and categorical (high vs. low) measure we examined the relationship between the anxiety-like behavior of virgin F2 females and the subsequent maternal behavior of these females. In addition, we quantified oxytocin (OTR) and vasopressin (V1a) receptor density within the lateral septum to determine the possible correlation with anxiety-like and maternal behavior. We find that, though activity levels within the open-field do predict latency to engage in pup retrieval, anxiety-like measures on this test are otherwise not associated with subsequent maternal behavior. OTR density in the dorsal lateral septum was found to be negatively correlated with activity levels in the open-field and positively correlated with frequency of nursing behavior. V1a receptor density was significantly correlated with postpartum licking/grooming of pups. Though we do not find support for the hypothesis that individual differences in trait anxiety predict variation in maternal behavior, we do find evidence for the role of OTR and V1a receptors in predicting maternal behavior in mice and suggest possible methodological issues (such as distinguishing between trait and state anxiety) that will be a critical consideration for subsequent studies of the anxiety–maternal behavior relationship.
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Introduction

It is commonly hypothesized that a causal relationship exists between anxiety and maternal care, based primarily on the co-occurrence of variation in these phenotypes. In humans, for example, anxious mothers are more likely to have insecure-resistant attached infants (Stevenson-Hinde et al., 2011). Higher maternal anxiety in primates has been associated with impaired infant retrieval, increased maternal rejection, and abuse of infants (Saltzman and Maestripieri, 2011; Troisi and D'Amato, 1994). Similarly, individual differences in maternal anxiety have been related to individual differences in primate maternal style (Altmann, 1980; Maestripieri, 1993; Troisi et al., 1991). Laboratory studies in rodents add support for this hypothesis, based on evidence that 1) spontaneous alloparental care and low levels of neophobia are both observed amongst juveniles (Lonstein and De Vries, 2000); 2) lactating dams exhibit both increased maternal care and reduced anxiety-like behavior compared to virgins (Fleming and Luebke, 1981; Lonstein, 2007); and 3) developmental disruptions in early life in rodents (as well as primates and humans) often lead to elevated anxiety levels and reduced or impaired parenting (Curley et al., 2009; Fleming et al., 2002; Maestripieri, 2005).

Investigations into the nature of the relationship between individual differences in anxiety-like and maternal behavior have typically compared the maternal characteristics of rodents bred selectively for anxiety-like behavior. For example, the Maudsley Reactive Strain of rats selected for high anxiety-like behavior in the open-field test show higher frequencies of nursing than the Maudsley Non-Reactive Strain (Holland, 1965). Likewise, Roman Low Avoidance rats selected for high anxiety-like behavior in a two-way avoidance task show higher frequencies of high-arched nursing than rats selected for low anxiety-like behavior (Driscoll et al., 1979; Fuemm and Driscoll, 1981). Amongst rats selectively bred for high anxiety-like behavior on an elevated plus maze (HABs), dams nurse pups more frequently, are more motivated to retrieve pups, and exhibit higher levels of maternal aggression compared to rats selected for low anxiety-like behavior (LABs) (Bosch, 2011; Neumann et al., 2005). Higher frequencies of arched nursing are also exhibited during the first week postpartum by HAB compared to LAB mouse dams (Kessler et al., 2011). Thus, amongst rodents selected for higher anxiety-like behavior there appears to be elevated levels of some maternal behaviors such as nursing. This appears to be contradictory to the prediction from

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human and primate work that higher anxiety would impair maternal care, but it must also be emphasized that each component of maternal behavior is likely to have its own unique relationship with anxiety.

The divergent conclusions reached by various studies of the link between anxiety-like and maternal behavior leaves the question of both the direction and nature of the anxiety–maternal care relationship an active area of research. One possible explanation for the contrasting findings within the existing literature may be methodological: approaches finding a positive association use selected lines whereas approaches finding a negative association rely on between-group variation. These methodological issues have implications for the study of the neurobiological basis of anxiety and maternal behavior as well as the neural mechanisms that may mediate any relationship between these behavioral phenotypes.

In the current study, we effectively combine both general methodological approaches that have been used to explore the link between anxiety and maternal care. We have previously shown that 129S and C57Bl/6J (B6) mice differ on measures of both anxiety-like and maternal behavior. 129S mice display high anxiety-like and limited exploratory behavior in an open-field test whereas B6 mice, in contrast, are highly exploratory in a novel environment and display reduced anxiety-like behavior (Curley et al., 2010). On measures of maternal behavior, we have found that lactating 129S females are slower to retrieve pups, lick pups less frequently, yet engage in a higher frequency of nursing behavior when compared to B6 dams (Champagne et al., 2007, 2009; Curley et al., 2010). In this study, we employed a reciprocal intercross breeding strategy between the F1 hybrid offspring of 129S and B6 mice similar to that commonly employed in QTL studies (Verhoeven et al., 2006). The purpose of this approach was to increase behavioral variability and to test whether the suggested relationship between anxiety-like and maternal behavior between inbred strains could also be observed within a genetically closed population comprised of the F2 offspring of these two progenitor strains. In addition, we explored the potential neurobiological substrates of this behavioral variation. Central oxytocin (OTR) and vasopressin 1a (V1a) receptors have been implicated in both anxiety-like behavior and social/maternal/reproductive behaviors (Donaldson and Young, 2008; Insel, 2010; Neumann, 2008) and thus may serve as substrate of the relationship between these distinct but potentially related behavioral phenotypes. Amongst inbred mice, a particularly high density of OTR and V1a receptors has been specifically quantified within the dorsal and ventral lateral septum compared to other rodents (Dubois-Dauphin et al., 1996; Insel et al., 1993). Given this species-specific receptor distribution and the fact that the lateral septum has been consistently hypothesized to be the forebrain region at which neuropeptides such as oxytocin and vasopressin may regulate both anxiety-like and maternal behavior (Bosch, 2011; Rotzinger et al., 2010), we proposed to investigate whether individual differences in dorsal and ventral lateral septum OTR and V1a receptor binding is indeed associated with natural variation in these behaviors.

Methodology

Animals and husbandry

All subjects used in this study were derived from C57BL/6J (B6) and 129S1/SvImJ (129S) laboratory mice (*Mus musculus*) which had been bred for over 20 generations in our own facility, the progenitors of which were brought in from Harlan UK. All animals were housed at the Sub-Department of Animal Behaviour at the University of Cambridge in accordance to the UK Home Office regulations. The animals were kept in opaque cages (42 cm × 12.5 cm × 12.5 cm) with steel wire lids on a reverse 12D:12L light cycle under a constant temperature of 21 °C and 55% humidity and provided ad libitum water and food (RM1 E rodent chow diet, Lillico, Surrey UK). All behavioral observations and tests took place during the dark period of the light cycle under dim red illumination.

Generation of F2 mice

For all matings, one male was housed in a cage with 2–3 females for 2 weeks. Females were singly housed at approximately 17–18 days of gestation. For the F1 generation, 3–5 month old B6 females were mated with 3–5 month old 129S males (producing F1-B6129S animals) and the reciprocal cross was also made such that 129S females were mated with B6 males (producing F1-129SB6 animals). These offspring were weaned at postnatal day (PN) 28 and housed in same sex groups of 3–4 prior to mating to produce the F2 generation. For the F2 generation, F1-B6129S and F1-129SB6 animals were inter-crossed in a two-by-two breeding design with a total of 60 F2 female offspring from 36 different mothers used for the current study. These females were weaned at PN28 and housed in same sex groups of 3–4 prior to behavioral assessment.

Behavioral testing procedure

All 60 F2 females were tested twice in the open-field. The first test occurred when the females were approximately 9 weeks of age and the second test occurred at 20 weeks of age. Immediately following the second open-field test, females were placed into a mating cage with an adult 129S breeder male. Females were singly housed at approximately 17–18 days of gestation. A majority of females successfully gave birth following mating, although several were re-mated with a novel male until pregnancy was achieved. Females did not differ in any aspect of their open-field behavior or maternal care with respect to the number of matings that they required to get pregnant.

Open-field test

The open-field test is a behavioral assessment of exploratory activity in an unfamiliar environment (reviewed in Prut and Belzung, 2003). The open-field used was a 90 cm × 90 cm × 60 cm plastic box. Females were confirmed to be in diestrus on the day of testing. On the day of testing, the mouse was removed from its home cage and placed directly into one corner of the open field. After a 10-minute session, the mouse was removed and returned to its home cage. Counts of fecal boli emitted in the open-field were assessed at this time. All testing was conducted under red (dark phase) lighting conditions (<5 lx). During analysis of the video recordings of testing sessions, the field was divided into a grid of 10 × 10 squares. Inner field exploration was defined as the time spent in the inner 9 × 9 squares, activity was defined as the number of square crossings, and pauses in movement within the field were defined as the duration of time spent immobile. Data was not obtainable for one female during test one and for two females during test two meaning that complete data for both tests existed for 57 females.

Pup retrieval test

On the day of birth, the lactating female and pups were removed from the home cage for approximately 10s and bedding was disturbed throughout the cage. Three pups from the litter were randomly placed away from the nest end of the cage, and the mother was then reintroduced. The latency to sniff a pup, retrieve the three pups, nestbuild and crouch over pups was recorded. The test was terminated at 15 min, resulting in a latency of 900 s for any behaviors not yet observed. Following testing, all pups and dams were weighed and returned to the home cage.