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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.

Assessment of postpartum maternal care

The procedure for assessing maternal behavior in mice has been previously reported (Champagne et al., 2007). Briefly, maternal behavior was scored from PN1 to PN6. Dams were observed in their home cage during the dark-phase of the circadian cycle under dim red light (<5 lx) and not disturbed for the duration of the 6-day observation period. Each day consisted of 4 observation periods, and each observation was 60 min in duration. Within each observation period, the behavior of each mother was scored every 3 min (20 observations/period × 4 periods per day = 80 observations/mother/day = 480 observations per mother over the 6 days). The following behaviors were scored: mother off pups, mother licking and grooming any pup, mother in nursing posture over pups, mother in contact with pups but not nursing, nestbuilding, self-grooming, eating and drinking. Females with fewer than 3 pups were excluded from the analysis. The highest frequency and variation in nursing and LG behavior is typically found on PN1. Therefore, measures of maternal care were assessed both across the entire first postpartum week, as well as on PN1.

OTR and V1a receptor autoradiography

Immediately following the last observation on day PN6, dams were sacrificed through rapid decapitation and brains extracted, placed briefly in isopentane, and stored at −80 °C. Eighteen F2 females were selected for inclusion in the receptor binding analysis. Females were rank-ordered based on their frequency of nursing from PN1–6 and every third brain taken. Females that were selected for brain analysis were not found to significantly differ from those females that were not selected on any behavioral measure (open-field or maternal) and were thus representative of the overall population. 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 OTR autoradiography using ^{125}I-d$(CH_2)_5$[Tyr-Me$)_2$,Tyr-NH_2^9] OVT (New England Nuclear, Boston, MA) and V1a receptor autoradiography using ^{125}I-lin-vasopressin [^{125}I-phenylacetyl -D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2] (New England Nuclear, Boston, MA) as previously described (Francis et al., 2000, 2002). 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. OTR binding was analyzed between Bregma +0.38 mm and −0.10 mm in the dorsal and ventral lateral septum. V1a receptor binding was analyzed between Bregma +0.50 mm and +0.14 mm in the dorsal and ventral lateral septum. For each animal, total and non-specific binding was measured for each region and the difference taken to yield specific binding which was converted to fmol/mg using radiolabelled microscales (GE Healthcare). The statistical analysis was performed on the mean of these values for each animal by brain region according to the mouse brain atlas (Paxinos and Franklin, 2003).

Statistical analysis

Associations between variables were tested using Pearson correlations without adjustment for multiple hypothesis testing (Perneger, 1998; Rothman, 1990; Savitz and Olshan, 1998). When data were not normally distributed, (pup retrieval only) Spearman rank correlations were used. Association between the frequency of nursing over the first week postpartum and open-field or receptor binding variables was tested using a multiple regression including litter size as a separate independent variable. Mediation analysis was conducted to test for possible directionality of relationships between highly inter-correlated variables. Data from the second open-field test (Time 2) was used in all analyses comparing open-field behavior to maternal behavior or receptor binding. Data from the final open-field test is typically considered to be more indicative of trait anxiety than data from the first open-field test (Time 1) (Adams and Geyer, 1986; Dulawa et al., 2004). Nevertheless, it should be noted that given the high degree of inter-correlation between behavioral variables at Time 1 and Time 2, all significant associations with maternal behavior and receptor binding detected using the open-field data from Time 2 could also be detected using data from Time 1. Group differences in maternal care between animals that showed 'high' or 'low' levels of behavior in the open-field was tested using independent t-tests. All statistics were implemented with SPSS 18.0.

Results

Individual differences in open-field behavior

The measures of activity and anxiety-like behavior in the open-field of F2 females are presented in Table 1. All measures were consistent across the two tests (Table 1 and Fig. 1). We found a high degree of inter-correlation amongst most open-field variables. For both runs of the open-field, time in the inner area was significantly positively correlated with squares crossed and both measures were significantly negatively correlated with time spent immobile (correlations between 0.58 and 0.90, all p values<0.001). The number of boli produced was positively correlated with time spent immobile and negatively correlated with time in the inner area and squares crossed.

Individual differences in maternal care

Summary statistics of retrieval behavior and postpartum maternal care are presented in Table 2 and Table 3. Of the 57 females who gave birth, one had fewer than 3 pups at birth and was eliminated from the retrieval analysis. For the remaining 56 females the average litter size at birth was 7.00 ± 0.29 pups (range = 3–13) and on PN1 litter size

Table 1
Stable individual differences in open-field behavior.

Measure	Run	Mean + SE	Minimum–maximum	Run 1 vs. Run 2 Pearson correlation	Run 1 vs. Run 2 paired *t*-test (df = 56)
Time immobile (s)	Run 1	132.0 ± 18.3	5–525	.72***	t = −2.72**
	Run 2	170.5 ± 21.7	8–584		
Time in inner area (s)	Run 1	56.5 ± 5.7	1–158	.78***	t = −2.11*
	Run 2	70.7 ± 9.2	0–252		
Squares crossed	Run 1	387.6 ± 29.0	14–891	.76***	t = 2.65**
	Run 2	339.8 ± 26.4	2–774		
Boli produced	Run 1	4.7 ± 0.3	0–12	.55***	t = −3.42***
	Run 2	5.6 ± 0.26	2–10		

Run1 n = 59, Run2 n = 58 (see Methodology).
*** p<0.001.
** p<0.01.
* p<0.05.

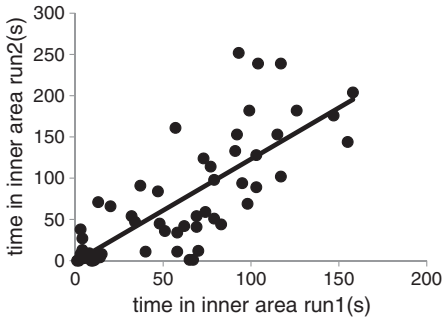

Fig. 1. Stability of individual differences in open-field exploration over time. Time spent in the inner area was found to be highly correlated amongst female mice tested at 9 weeks of age and re-tested at 20 weeks of age.

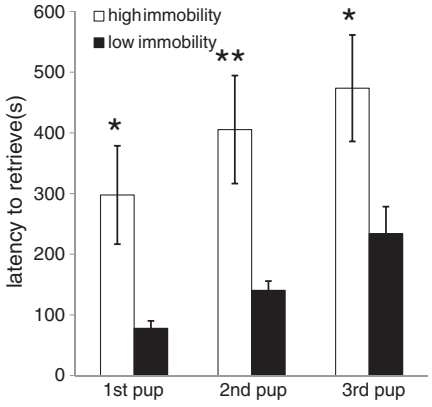

Fig. 2. Group differences (mean ± sem) in frequency of immobility during open-field testing predicts latency to retrieve pups. *p<.05, **p<.01.

was significantly positively related to the frequency of eating (r = .35, p<.05), but was not related to the frequencies of any other postpartum behavior. Across the first week postpartum (PN1–PN6), females with larger litters were found to spend more time eating (r = .59, p<.001) and drinking (r = .29, p<.05) and less time nursing (r = −.49, p<.001). During the first week postpartum, the frequency of nursing was significantly negatively correlated with the frequency of eating (r = −.79, p<.001) and drinking (r = −.31, p<.05). Mediation analysis revealed that the relationship between litter size and nursing frequency was fully mediated by eating frequency (% mediation = 93%; Sobel test z = 4.19; p<0.0001). The frequency of licking/grooming was not related to litter size or the frequency of any other postpartum behaviors.

Relationship between retrieval behavior and postpartum maternal care

No significant correlation was found between the rank order performance for a female on any measure of the retrieval test and the frequency of any postpartum care behavior over either the whole week postpartum or on PN1.

Relationship between open-field behavior and maternal care

We compared maternal care (PN0 retrieval, PN1 maternal behavior, and average maternal behavior across the first week postpartum) with measures from the Time 2 run of the open-field.

Open-field behavior and pup retrieval

The time spent immobile in the open-field was significantly related to the latency to retrieve the first pup (r = .283, p<.05), however, this measure was only weakly predictive of the latency to retrieve the second (r = .192, p = .16) or third pups (r = .176, p = .20). We also found that latency to retrieve the first pup was marginally related to the number of squares crossed (r = −.225, p = .10) and time spent in the inner area (r = −.221, p = .11). No other significant relationships were found in this analysis.

In a second approach to this analysis, F2 females were categorized as 'high' or 'low' for each open-field behavior if their score was above or below the upper and lower quartile range. Females who were classified as high in immobility were found to be significantly slower to retrieve all three pups (1st − $t_{1,25}$ = 2.58, p<.05; 2nd − $t_{1,25}$ = 2.83, p<.01; 3rd − $t_{1,25}$ = 2.38, p<.05 − Table S1, Fig. 2). Females that were classified as high on the number of squares crossed were found to be marginally faster to retrieve the first pup ($t_{1,25}$ = 1.72, p<.10).

Open-field behavior and postpartum maternal care

We found no evidence of a relationship between the frequency of nursing or licking/grooming on either PN1 or across the first week postpartum with any measure of behavior in the open-field. Additionally, even after controlling for litter size, nursing remained unrelated to open-field behavior. F2 females grouped as 'high' vs. 'low' for each open-field behavior were also indistinguishable in their postpartum maternal care. (Table S1).

Relationship between receptor binding and behavior

OTR binding

OTR binding in the dorsal lateral septum was significantly positively related to time spent immobile (r = .577, p<.05) and negatively related to the number of squares crossed (r = −.689, p<.005; see Fig. 3) but not related to time in the inner area or number of boli produced during open-field testing. OTR binding in the ventral lateral septum was not related to any measure in the open-field and retrieval behavior was not associated with OTR binding in the dorsal or ventral lateral septum.

Table 2
Summary of behavior latencies in a pup retrieval task (n = 56).

	Sniff pup (s)	Retrieve 1st pup (s)	Retrieve 2nd pup (s)	Retrieve 3rd pup (s)	Nestbuild (s)	Crouch over pups (s)
Median and IQRs	4.5 [3.0/10.0]	84.5 [46.8/261.0]	152.5 [95.0/403.5]	212.0 [126.0/631.8]	337.0 [226.5/648.0]	656.5 [389.5/900.0]

IQR — interquartile range.

Table 3
Frequencies of postpartum behaviors (n = 56).

	Nursing	Licking/grooming	Nestbuilding	Self-grooming	Eating	Drinking
PN1 Median ± sem	58.8 ± 2.3	9.7 ± 0.7	8.6 ± 0.9	1.8 ± 0.3	16.5 ± 1.0	0.9 ± 0.1
PN1-6 Median ± sem	53.1 ± 1.4	6.9 ± 0.3	6.5 ± 0.5	2.5 ± 0.2	21.9 ± 0.8	1.3 ± 0.1

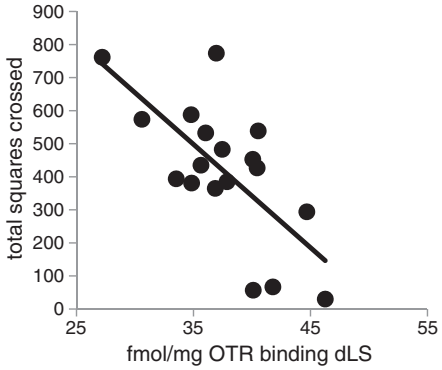

Fig. 3. Correlation between OTR binding in the dorsal lateral septum (dLS) and total squares crossed during open-field testing.

In both the dorsal and ventral lateral septum there was a significant positive correlation between OTR binding and the frequency of nursing on day PN1 (dorsal — $r = .508$, $p < .05$; ventral — $r = .573$, $p < .05$; Fig. 4A). Nursing frequency over the first week postpartum was positively related to the density of OTR in the ventral lateral septum after controlling for litter size ($\beta = .405$, $t = 2.18$, $p < 0.05$; Fig. 4B), and together litter size and receptor binding accounted for 49% of variance in frequency of nursing ($R^2 = 0.49$, $F_{2,\ 17} = 7.09$, $p < 0.01$). Levels of OTR binding in the dorsal lateral septum were not related to nursing over the first week postpartum. Furthermore, levels of OTR in both regions do not predict the frequency of postpartum licking/grooming.

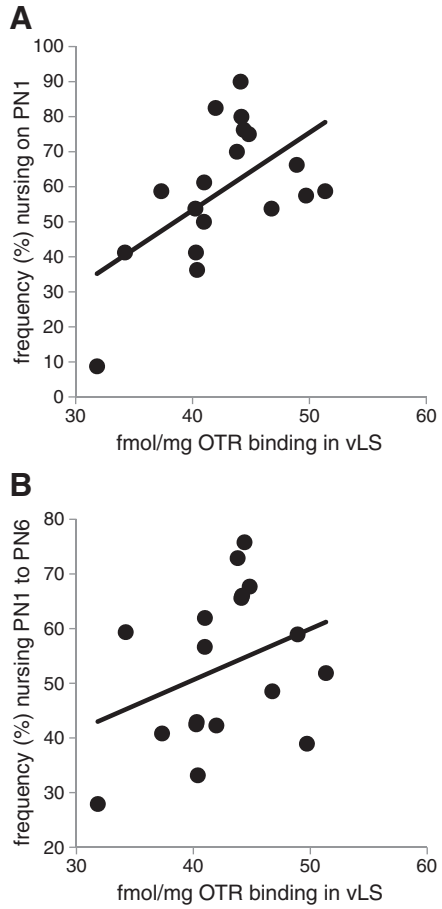

Fig. 4. Correlation between OTR binding in the ventral lateral septum (vLS) and A) frequency of nursing on PN1 and B) frequency of postpartum nursing on PN1–PN6.

V1a receptor binding

Levels of V1a receptor binding in the lateral septum (dorsal or ventral) were not associated with individual differences in any measure of the open-field or retrieval test. In the dorsal lateral septum there was a trend for the levels of V1a receptor binding to be related to the frequency of nursing on day PN1 ($r = .456$, $p = .057$; Fig. 5A) but not over the first week postpartum. Levels of V1a receptor binding in the ventral lateral septum were found to be unrelated to the frequency of postpartum nursing. In contrast, V1a receptor binding in the ventral lateral septum was significantly positively correlated with the frequency of licking/grooming over the first week postpartum ($r = .544$, $p < .05$; Fig. 5B). There was no relationship found between the levels of V1a receptor binding in the dorsal lateral septum and licking/grooming behavior.

Discussion

In the current study we have used within-group and between-group approaches to examine the relationship between maternal and anxiety-like behavior in laboratory mice and have examined the potential role of OTR and V1a receptors within the lateral septum in these behavioral phenotypes. Using both a continuous (correlational) and categorical (group selection for "high" or "low" anxiety-like behavior) analysis, we find limited support for a relationship between anxiety-like and maternal behavior. Though the cohort of F2 female mice used in these studies were generated from phenotypically divergent inbred strains, displayed a high degree of behavioral variation, and exhibited stable individual differences in open-field behavior, we found that classic indices of anxiety-like behavior, such as the number of boli produced or time spent in the inner area, were not predictive of subsequent maternal care. Consistent with this finding,

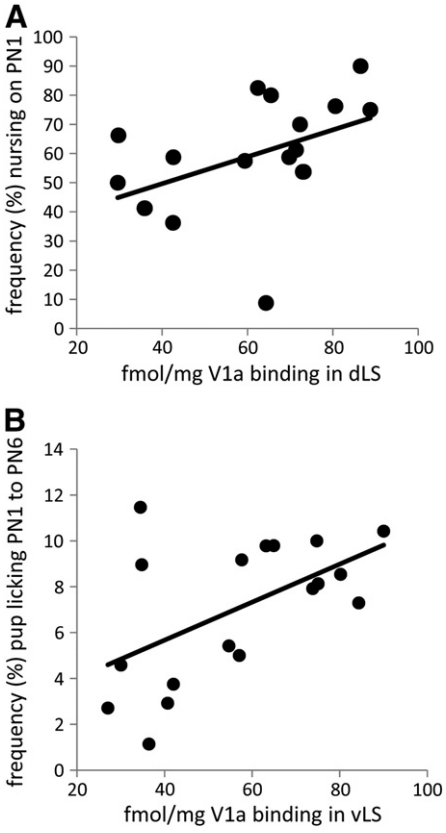

Fig. 5. Correlation between V1a receptor binding in the A) dorsal lateral septum (dLS) and nursing frequency on PN1 and in the B) ventral lateral septum (vLS) and postpartum licking/grooming (PN1–PN6).

analysis of OTR and V1ar receptor binding in the dorsal and ventral lateral septum indicates a significant positive correlation with postpartum nursing and licking/grooming of pups respectively with limited predictive utility for measures of anxiety-like behavior in the open-field.

The common assumption of a causal relationship between anxiety and maternal care (whereby increased or decreased anxiety leads to decreased or increased maternal care) is derived from studies examining how group differences in state anxiety predicts group differences in mother–infant interactions. For example, lactating female rats exhibit reduced anxiety-like behavior compared to virgin females and it has been hypothesized that this behavioral change may facilitate the onset of maternal care by aiding the female to overcome the neophobia elicited by pups (Fleming and Luebke, 1981; Lonstein, 2007; Lonstein and De Vries, 2000). Likewise, the facilitation of pup retrieval in virgin adult female rodents by infusion with prolactin, estrogen and oxytocin have been suggested to be mediated in part by reducing state anxiety and neophobia of pups (Numan and Insel, 2003). In considering these previous findings in the context of the current study, perhaps an important issue is the distinction between state and trait anxiety. Though it may certainly be the case that manipulations (hormonal, pharmacological or experiential) which reduce state anxiety will facilitate maternal care, the results of our study would suggest that the enduring characteristic trait anxiety of individuals is not predictive of subsequent maternal behavior. Moreover, our analysis of this trait anxiety requires the characterization of behavior on a continuum such that that the correlation at an individual level of analysis can be established. The current study is one of the first to implement such a research design to directly assess how individual rather than selectively bred strain differences (Bosch and Neumann, 2008) in trait anxiety-like behavior may predict future maternal care.

The level of activity displayed by virgin female mice during open-field testing was the only measure revealed in our analysis to predict any behavior of postpartum females. However, this measure was only predictive of pup retrieval and was not significantly correlated with frequency of postpartum mother–infant interactions. An important issue to consider when establishing the link between anxiety and maternal behavior is the multifaceted nature of both of these behaviors. In the case of maternal behavior, the current study finds no association between postnatal pup retrieval and frequency of postpartum mother–infant interactions, a finding consistent with our previous work in inbred and outbred mouse strains (Champagne et al., 2007), suggesting that these measures are assessing distinct aspects of the maternal response. Like most complex behaviors, maternal care is the product of sensory, motivational, and arousal processes as well as distinct behaviors that allow interactions with pups. Moreover the approach used to assess maternal care will likely assess distinct facets of the behavioral responsiveness to pups. In the case of the pup retrieval test, dams are briefly removed from the home cage and the bedding within the cage is disturbed prior to behavioral assessment. As such, there is an aspect of novelty-induced neophobia inherent in subsequent behavior thus creating similarities between this measure and tests of anxiety-like behavior such as the open-field.

Oxytocin (OT) has an established role in both the onset and maintenance of maternal care, though it should be noted that the majority of this research has been carried out in rats or sheep (Kendrick et al., 1997; Neumann, 2008). Within the lateral septum, release of OT occurs in response to suckling (Landgraf et al., 1991; Neumann and Landgraf, 1989). In rats, OTR mRNA has been found to increase in the lateral septum during late pregnancy (Young et al., 1997), though it is questionable whether this increase is sustained following parturition (Housham et al., 1997; Insel, 1990; Meddle et al., 2007). Despite the high levels of OTR in the lateral septum of mice, there has been limited investigation of the role of this neuropeptide in mouse maternal behavior. Indeed, any role for central OT in regulating the onset and maintenance of maternal care in mice has been questioned by the data from OT and OTR knockout models. OT and OTR knockout mice have impaired milk let-down but exhibit normal parturition and maternal behaviors (Macbeth et al., 2010; Nishimori et al., 1996; Young et al., 1996), though some moderate deficits in parental behaviors have also been reported (Pedersen et al., 2006; Ragnauth et al., 2005; Takayanagi et al., 2005). However, amongst *CD38* knockout mice that have deficits in OT release, maternal behavior can be restored following administration with OT (Jin et al., 2007) and virgin or pregnant female mice that receive central administration of OT show reduced levels of infanticide (McCarthy, 1990). Further, *Peg3* knockout mice have a number of maternal care impairments and have reduced OTR binding in the MPOA and lateral septum (Champagne et al., 2009). Thus, as in other mammalian species, the oxytocinergic system may be important for regulating maternal behavior in mice.

In the current study, we found the frequency of OTR binding in the ventral lateral septum to be positively related to the frequency of nursing exhibited by lactating females over the whole first week postpartum. We also found that in both the dorsal and ventral lateral septum, the levels of OTR binding were strongly related to the frequency of nursing on PN1. These findings are consistent with research indicating that levels of OT acting on OTR in the lateral septum are related to the facilitation of milk let-down and maintenance of nursing in response to suckling. Though the frequency of nursing and licking/grooming are highly correlated on PN1, we found no significant association between OTR binding with the levels of licking/grooming on PN1, suggesting a degree of specificity between septal OTR and nursing behavior. In previous studies, individual differences in OTR binding in the ventral lateral septum were found to be positively correlated with licking/grooming behavior in rats (Champagne et al., 2001; Francis et al., 2000), and amongst juvenile female prairie voles, latency to adopt a crouch over pups was found to be negatively associated with levels of OTR binding in the lateral septum (Olazabal and Young, 2006). Taken together, these studies suggest that species specific variation in OTR binding in the lateral septum may be a neurobiological substrate of individual differences in responsiveness to pups.

Vasopressin (AVP) has long been recognized as a neuropeptide that is central to the regulation of social and aggressive behaviors, as well as paternal behavior in biparental species (Caldwell et al., 2008). Pharmacological manipulation of V1a receptors in the lateral septum has illustrated the importance of these receptors in promoting paternal care in voles (Wang et al., 1994). Recently, there has been an increasing acknowledgement of the role of vasopressin in coordinating maternal behavior. For example, AVP mRNA expression is up-regulated in the PVN and AVP is released into limbic areas such as the ventral lateral septum at parturition (Bosch et al., 2007; Landgraf et al., 1991; Neumann et al., 1993). Moreover, arched back nursing in lactating rats can be stimulated following an icv infusion of AVP whereas the injection of V1a receptor antagonists can attenuate this form of maternal care (Bosch and Neumann, 2008). Further, AVP also appears to be a potent modulator of maternal aggression in rats (Bosch and Neumann, 2010; Nephew et al., 2010). Fewer studies have examined the role of AVP in mice, though central acute infusion of AVP increases arched-back nursing and pup retrieval in LAB mice but does not appear to have any effect in HAB mice (Kessler et al., 2011).

Here we report a very strong association between V1a receptor binding in the ventral lateral septum and postpartum licking/grooming with no association detected between V1a binding in this region and frequency of nursing across the first week postpartum. These data suggest that AVP acting on V1a receptors in the mouse ventral lateral septum could well be part of a maternal circuit coordinating appetitive maternal behaviors such as licking/grooming. This would be congruent with the extensive AVP innervation of the mouse lateral septum with neurons originating from the BNST and amygdala (Caffe

et al., 1987; Rood and De Vries, 2011). Furthermore, in the mouse, when compared with the rat, there is far more extensive evidence of AVP fibers in regions associated with appetitive behaviors such as the nucleus accumbens, VTA and dorsal raphe nucleus (Rood and De Vries, 2011). This could be suggestive that appetitive maternal behaviors in the mouse may be as reliant upon vasopressin as upon oxytocin.

Previous pharmacological and physiological studies have established a role for the actions of AVP and OT in the lateral septum in regulating anxiety-like behaviors (Engelmann et al., 2000; Everts and Koolhaas, 1999; McCarthy et al., 1996; Rotzinger et al., 2010). In this study, we did not find any evidence for an association between any measure of anxiety-like behavior in the open-field (boli produced and time in inner area) and the binding density of septal OTR or V1a receptors. Most previous work exploring how OT and AVP are involved in the regulation of anxiety-like behavior has focused on rats and therefore it is possible that in the mouse the density of OTR and V1a receptors in the lateral septum are not functionally relevant in mediating individual differences. However, it is also possible that the role of these septal receptors may be contextually dependent and such a relationship may be found if anxiety-like behavior was assessed in other experimental paradigms. Finally, we did find evidence of a significant negative association between OTR binding in the dorsal lateral septum and open-field locomotor activity. Interestingly, lesions to the dorsolateral part of the lateral septum lead to decreased locomotor activity in the open-field (Lee et al., 1988). While pharmacological manipulation of septal V1a receptors do not appear to modulate activity levels (Liebsch et al., 1996), little is currently known about the role of OT in this brain region with regard to this behavior.

Conclusions

In this study we fail to find support for the hypothesis that individual differences in anxiety are predictive of postpartum maternal behavior. Establishing the nature and direction of the anxiety–maternal behavior relationship is challenging and though there may be species-specific issues, methodological considerations may certainly reconcile the current findings with previous literature on this topic. Moreover, whereas reductions or elevations in state anxiety may predict increases or decreases in maternal behavior, individual differences in trait anxiety may not yield similar findings. However, this study does provide support for the role of OTR and V1a receptors in the lateral septum in regulating mouse maternal behavior. We find evidence that there appears to be discrete roles for each neuropeptide within the lateral septum, with variation in nursing and licking/grooming frequency being related to the levels of OTR and V1aR binding respectively. This is one of the first studies to demonstrate that variation in OTR and V1aR binding relates to individual differences in mouse maternal care and suggests that each species may be unique with regard to how these neuropeptides are utilized to facilitate maternal care.

Supplementary materials related to this article can be found online at doi:10.1016/j.yhbeh.2012.01.013.

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