

Naturally Occurring Differences in Maternal Care are Associated with the Expression of Oxytocin and Vasopressin (V1a) Receptors: Gender Differences

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Abstract

Variations in maternal care have been associated with long-term changes in neurochemistry and behaviour in adult rats. Rats receiving high levels of licking and grooming as pups are less fearful and more maternal than rats receiving low levels of maternal licking and grooming. Central pathways for oxytocin and vasopressin have been implicated in the neurobiology of anxiety and social behaviours. We assessed whether variations in maternal care were associated with differences in oxytocin receptors (OTR) or vasopressin (V1a) receptors in the brains of adult offspring. In the central nucleus of the amygdala and bed nucleus of the stria terminalis, OTR binding was increased in adult females, but not adult males, that had received high levels of maternal licking and grooming as pups. Conversely, amygdala V1a receptor binding was increased in males, but not females, that had received high levels of maternal licking and grooming. These findings suggest that variations in maternal care may influence the expression of oxytocin and vasopressin receptors in a gender-specific manner.

Naturally occurring variations in maternal care in the rat appear to permanently alter behaviour and neurochemistry in adult offspring (1, 2). Male pups that receive high levels of maternal licking and grooming (high LG), develop into adults with lower levels of fear and anxiety relative to those that received low levels of maternal licking and grooming (low LG). These high LG male offspring have been reported to show several neurochemical changes, including greater benzodiazepine receptor binding in the amygdala and lower corticotropin releasing hormone receptor binding in the locus coeruleus compared to low LG offspring (2). Recently, adult females receiving increased maternal licking and grooming in infancy were reported to provide increased maternal care to their own offspring compared to low LG females (3). These high LG females have increased oxytocin receptor (OTR) binding in various brain regions compared to low LG females (4). Because oxytocin is important for the initiation of maternal care in several mammalian species (5),

these findings suggested a possible link between rearing experience, oxytocin receptors and maternal care.

The current study was designed to assess whether variations in maternal care are associated with individual differences in OTR binding in male as well as female rats. Because intact adult male rats do not naturally exhibit parental behaviour, we hypothesized that rearing effects on OTR binding would be greater in females than in males. There is evidence from both mammalian (6) and nonmammalian (7) vertebrates that the related nine amino acid peptide, arginine vasopressin, or its ancestral form, arginine vasotocin, may be important for male-specific behaviours, such as paternal care, territorial behaviour, aggression and male mating behaviour. To investigate the possibility that rearing effects in males may be evident in vasopressin rather than oxytocin neurotransmission, we compared binding to vasopressin (V1a) receptors in the brains of high LG and low LG males and females in adulthood.

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Materials and methods

Animals

Animals were male and female adult offspring reared by Long-Evans female rats (obtained from Charles River Canada, St Constant, Quebec, Canada) characterized as high or low LG dams. At weaning, rats were housed in same-sex Plexiglass cages with food and water available ad libitum. The colony was maintained on a 12:12 light:dark cycle with lights on at 08.00 h. Upon adulthood (no earlier than 100 days of age), male and female rats were sacrificed and the brains obtained for receptor autoradiography.

Maternal behaviour

Maternal behaviour was characterized using a modified version of Meyers *et al.* (8). The behaviour of each dam was observed beginning on the day following birth of the litter. Rats were observed for five 75-min observation periods daily for the following 8 days. There were three observation periods during the light phase and two periods during the dark phase of the light:dark cycle (25 observations/period \times 5 periods/day = 125 observations/mother/day). Within each observation period, the behaviour of each mother was scored every 3 min for the following: mother off pups, mother carrying pup, mother licking and grooming any pup, mother nursing pups in either an arched-back posture, a 'blanket' posture in which the mother lays over the pups, or a passive posture in which the mother is lying either on her back or side while the pups nurse. Behavioural categories were not mutually exclusive. For example, licking/grooming most often occurred while the mother was nursing pups. The data were scored as the percentage of total observations during which the animals were engaged in the target behaviour. High LG mothers were defined as females whose frequency scores for licking and grooming were 1 SD above the mean. Low LG mothers were defined as females whose maternal licking and grooming frequencies were 1 SD below the mean.

Receptor autoradiography

To avoid litter effects, brains were obtained from one male and one female offspring of each high or low LG litter ($n=4-5$ per group). Following decapitation, brains were quickly removed, frozen in -70°C isopentane, and stored at -80°C . Brains were sectioned 20 μm thick, mounted onto Permafrost slides and stored at -80°C until the time of assay. For OTR binding, [^{125}I]-ornithine vasotocin analogue [(^{125}I)OVTA] was employed [vasotocin, $\text{d}(\text{CH}_2)_2[\text{Tyr}(\text{Me})^2, \text{Thr}^4, \text{Orn}^8, (^{125}\text{I})\text{Tyr}^9\text{-NH}_2]$; 2200 Ci/mmol]; (NEN Nuclear, Boston, MA, USA). For V1a receptor binding, [^{125}I]-lin-vasopressin [^{125}I -phenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH $_2$]; (NEN Nuclear) was used.

Sections were allowed to thaw to room temperature (RT) and then immersed in 0.1% paraformaldehyde for 2 min to optimize tissue integrity. Sections were then rinsed three times in 50 mM Tris-HCl (pH 7.4) at RT for 5 min and incubated for 60 min at RT in a solution of 50 mM Tris-HCl (pH 7.4) with 10 mM MgCl $_2$, 0.1% bovine serum albumin, 0.05% bacitracin, and 50 μM [^{125}I]-lin-vasopressin or 50 μM [^{125}I]-OVTA. Non-specific binding was determined by incubating adjacent sections with the radioactive specific ligand as well 50 μM of unlabelled Thr $_4$, Gly $_7$ oxytocin, a selective oxytocin ligand (Peninsula Laboratories, Belmont, CA, USA) or 50 μM of unlabelled [1-(β -mercapto- β , β -cyclo-pentamethylene propionic acid),2-(O-methyl)-tyrosine]-arg $_8$ -vasopressin, selective for the V1a receptor. Following incubation, sections were washed 4 \times 5 min in 50 mM Tris-HCl (pH 7.4) with 10 mM MgCl $_2$ at 4 $^\circ\text{C}$, followed by a final rinse in this same buffer for 30 min. Slides were then quickly dipped in cold dH $_2$ O and rapidly dried with a stream of cold air.

Sections were apposed to Kodak BioMaxMR film (Kodak, Rochester, NY, USA) with [^{125}I] microscale standards for 72 h. Autoradiographic [^{125}I]-receptor binding was quantified from film using the NIH Image program (<http://rsb.info.nih.gov/nih-image/>). An average of nine sections per animal per area were scored using the Paxinos and Watson rat atlas (9) as a reference. For amygdala sections, adjacent slides were stained with acetylcholinesterase to aid in identification of the central nucleus (Fig. 1).

Statistical analysis

Data are represented as mean \pm SE. Non-specific binding was subtracted from total binding to yield values for specific binding. Because slides from males and females were assayed separately, high LG and low LG means were compared for each gender independently using Student's *t*-test for each region.

Results

^{125}I -OVTA binding

Specific binding of [^{125}I]-OVTA was evident in the ventromedial nucleus of the hypothalamus, central nucleus of the amygdala and the bed nucleus of the stria terminalis, as previously described (10), in both females and males. In females, specific binding of [^{125}I]-OVTA was increased in the high LG group relative to the low LG group in the bed nucleus of the stria terminalis ($P<0.01$) and in the central nucleus of the amygdala ($P<0.05$; Figs 2 and 3). There was no group difference in the ventromedial nucleus of the hypothalamus.

In males, no significant differences in [^{125}I]-OVTA binding were observed between high or low LG animals in any of the brain regions examined (Figs 2 and 3).

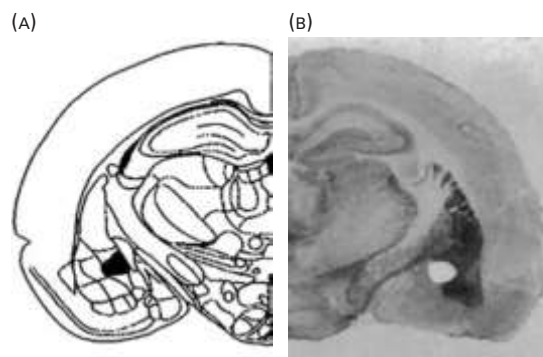


FIG. 1. A schematic demonstrating the region of the amygdala scored as the central nucleus. Panel (A) is from Paxinos and Watson (9) and (B) is a representative acetylcholinesterase stain used to identify individual nuclei within the amygdala. In (A), the central nucleus of the amygdala is indicated in black and in (B) it is indicated in white.

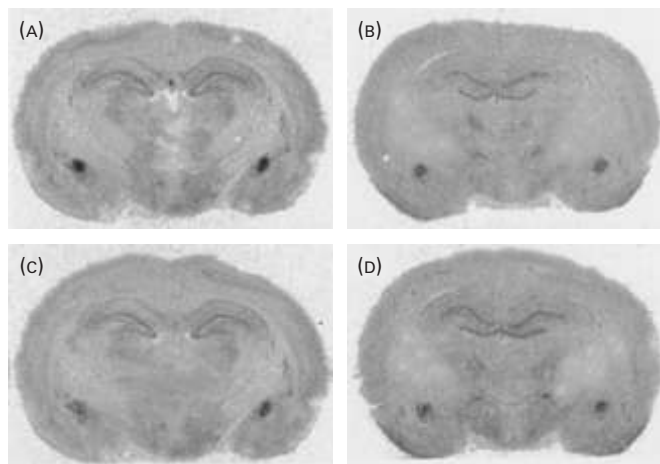


FIG. 2. Oxytocin receptor autoradiography in the central nucleus of the amygdala of female (A,B) and male (C,D) high (A,C) and low licking and grooming (B,D) animals. Significant differences in oxytocin receptor expression were detected in the central nucleus of the amygdala ($P<0.05$) of female, but not male, animals.

¹²⁵I-linear-vasopressin binding

Specific binding of ¹²⁵I-linear-vasopressin was found in the lateral septum and central nucleus of the amygdala, as previously reported (11). In females, no significant binding differences were observed between high and low LG groups in either the lateral septum or the central nucleus of the amygdala (Fig. 5).

In contrast to the absence of a treatment effect on ¹²⁵I-linear-vasopressin binding in females, a significant effect was observed in the central nucleus of the amygdala ($P < 0.05$) of males, with greater ¹²⁵I-linear-vasopressin binding in the high LG male animals compared to the low LG males (Fig. 5). No significant effect was observed in the lateral septum.

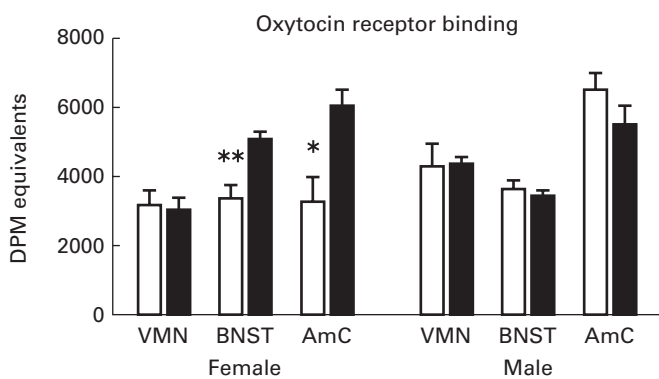


FIG. 3. Quantitative analysis of oxytocin receptor binding in select regions of high and low oxytocin animals. Note the significant group differences in the bed nucleus of the stria terminalis (BNST) (** $P < 0.01$) and central nucleus of the amygdala (AmC) (* $P < 0.05$) in female, but not male, animals. VMN, ventromedial nucleus of the hypothalamus.

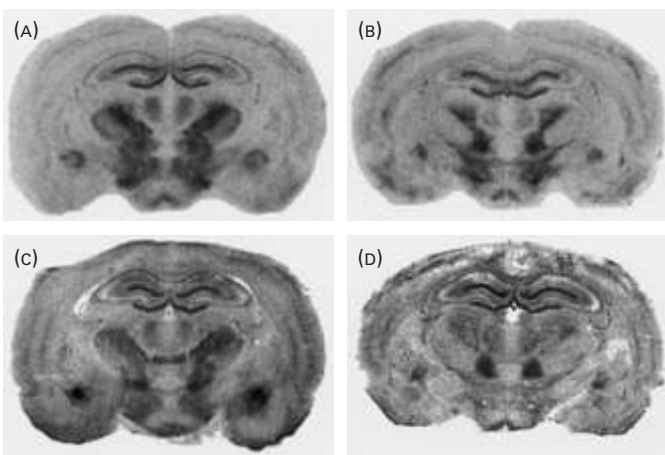


FIG. 4. V1a receptor autoradiography in the central nucleus of the amygdala in female (A,B) and male (C,D) high (A,C) and low licking and grooming (B,D) animals. Significant differences in V1a receptor expression were detected in the central nucleus of the amygdala (* $P < 0.05$) of male, but not female, animals.

Discussion

The current study confirms that variations in maternal behaviour are associated with differences in binding to brain oxytocin receptors; however, this association is gender-specific. High LG was associated with a 40% increase in OTR binding, but only in female and not male offspring. Conversely, High LG was associated with increases in V1a receptor binding in male but not female offspring. This double dissociation was found specifically in the central nucleus of the amygdala, such that differences in binding in this nucleus were observed for OTR in females and V1a receptors in males.

This study is limited by the use of a single concentration for binding, so we cannot conclude that changes in binding reflect a change in receptor number rather than a change in affinity. It is also possible that changes in receptor binding occurred elsewhere in the brain or periphery; our analysis was limited to the hypothalamus, bed nucleus of the stria terminalis, and amygdala. However, within these areas, the differences in binding were consistent across litters chosen for differences in maternal care, with males and females derived from the same litters.

How could differences in maternal care confer enduring changes in OTR and V1a receptors? Because grooming has been linked to oxytocin release in adults (12), it might be hypothesized that maternal grooming releases oxytocin in the pup, leading to a lasting increase in OTR expression. An analogous process by which other neurotransmitters in development confer long-term changes in receptors has been described as chemical imprinting (13). However, in the adult rat brain, OTR binding appears remarkably resistant to decreases and relatively resistant to increases in oxytocin (14). Oxytocin release in development may have more enduring effects (15), but we know of no evidence that grooming releases oxytocin centrally in pups or that the effects of grooming on oxytocin release are different in males and females.

In contrast to their relative resistance to changes in oxytocin, OTRs in the rat brain are exquisitely sensitive to

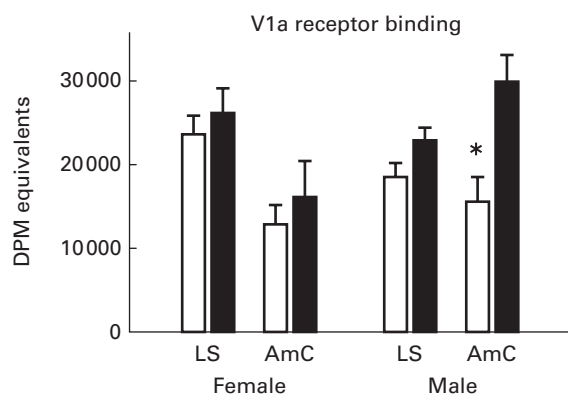


FIG. 5. Quantitative analysis of V1a receptor binding in the lateral septum (LS) and central nucleus of the amygdala (AmC) of high and low licking and grooming animals. Note the significant group differences in male, but not female, animals in the AmC (* $P < 0.05$).

changes in gonadal steroids (16, 17). If differences in maternal behaviour affect circulating oestrogen or testosterone differently in male and female pups then OTR expression might be influenced in a gender-specific fashion. While differences in perinatal levels of testosterone have been implicated in several morphological and neurochemical sexual dimorphisms in the rat brain (18), the effects of gonadal steroids on OTR expression have been most evident in the VMH and not the central nucleus of the amygdala (17). We observed effects of differential LG in the central nucleus of the amygdala and not the ventromedial nucleus of the hypothalamus. In fact, OTR binding in the subiculum (and central nucleus of the amygdala) appears to be responsive to changes in adrenal steroids (19). A more likely explanation is that maternal stimulation alters circulating corticosterone (20), thereby inducing OTR in the central nucleus of the amygdala. It is not clear, however, why such an effect is permanent or why this effect would be observed only in females.

Conversely, the mechanism by which maternal care might alter V1a receptor binding exclusively in males is unclear. Although V1a binding is relatively insensitive to increases in vasopressin (21), there is some evidence that vasopressin administered during development may have long-term effects on receptors (22, 23). Unlike OTRs, V1a receptors are not responsive to changes in gonadal steroids, but they appear to be sensitive to changes in glucocorticoids (24, 25). Thus, if increased levels of maternal stimulation altered circulating levels of corticosterone, one might observe changes in V1a receptor expression. Again, it is not obvious why such an effect would be observed only in males, but there is clearly a sexual dimorphism in the level of vasopressin, which can be observed developmentally (26, 27). These findings suggest that the development of the vasopressin system follows a different time-course in males and females, and perhaps this may affect the development of the V1a receptor system.

The observed gender dimorphic effects on OTR and V1a receptors are consistent with studies across several species which demonstrate more prominent effects of vasopressin in males and more significant effects of oxytocin in females (6). Indeed, such gender differences have even been reported in the ancestral forms of both nonapeptides. In the midshipman fish, males respond behaviourally to administration of vasotocin (a vasopressin-like peptide) while females only respond behaviourally to isotocin (an oxytocin-like peptide) and not vasotocin (7). Similarly, in prairie voles, pair bonding appears to be influenced more by oxytocin in females and vasopressin in males (28, 29). Whereas oxytocin facilitates the onset of maternal behaviour (5), vasopressin has been reported to induce paternal behaviour in a biparental rodent (30).

The differences in amygdala receptors may be related to previously reported differences in the behaviour of high and low LG offspring (1–3). Oxytocin administered to the amygdala has anxiolytic effects in females (31), although this may not be evident in male rats (32). As adults, high LG females spend more time exploring the inner area of an open-field compared to low LG females (3, 4). High LG females also subsequently become high LG mothers with their own litters (4). Male offspring subjected to different rearing environments also differ in their expression of fear (1, 2). Although these behavioural effects have been previously related to

changes in central benzodiazepine and corticotropin releasing hormone systems (2), it is also possible that rearing effects on maternal and anxiety-related behaviours are influenced by changes in OTR or V1a receptors.

In conclusion, our results demonstrate that naturally occurring differences in maternal care in the rat are associated with differences in neuropeptide receptor binding in the brains of adult offspring. These effects were different in male and female offspring. In female, but not male offspring, increased maternal licking and grooming received early in development was associated with greater oxytocin receptor binding in the bed nucleus of the stria terminalis and the central nucleus of the amygdala. Male but not female high LG offspring have greater V1a receptor binding in the central nucleus of the amygdala compared to low LG offspring. The mechanism by which maternal stimulation regulates neuropeptide receptor expression in offspring has yet to be elucidated, but these results demonstrate that the effects are sexually dimorphic and are evident in systems previously implicated in the expression of social behaviour.

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