Behavioral Neuroscience
2001, Vol. 115, No. 6, 1349–1356

0735-7044/01/$5.00 DOI: 10.1037//0735-7044.115.6.1349

Day Length and Sociosexual Cohabitation Alter Central Oxytocin Receptor Binding in Female Meadow Voles (*Microtus pennsylvanicus*)

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In voles (*Microtus*), central oxytocin (OT) receptor patterns are associated with interspecific social organization. Social, monogamous voles have more OT receptors in the extended amygdala than asocial, nonmonogamous voles. Nonmonogamous meadow voles (*Microtus pennsylvanicus*), which exhibit seasonal changes in social organization (long day [LD] females are territorial, short day [SD] females live socially), provide a model for examining whether OT receptor patterns are associated with seasonal changes in intraspecific social behaviors. The authors examined whether sexually inexperienced (naive) SD females had more OT receptor binding than naive LD females. Naive SD females had greater OT receptor binding in the lateral septum (LS), lateral amygdala (LatAmyg), and central amygdala (CenAmyg) than less social, naive LD females. Because both SD and LD females acquire partner preferences, the authors assessed whether OT receptor binding was associated with partner preference onset. For LD females, partner preference onset corresponded with greater OT receptor binding in the anterior olfactory nucleus, LS, and bed nucleus of the stria terminalis, compared with naive LD females. In contrast, naive SD females and those exhibiting partner preferences did not differ. However, SD females that failed to acquire partner preferences showed less OT binding in the LatAmyg and CenAmyg. This study is the first to show that central OT receptor patterns are associated with seasonal changes in intraspecific social organization and partner preference onset in a nonmonogamous rodent.

During the summer breeding season, free-living male meadow voles typically maintain diffuse ranges that encompass the mutually exclusive territories of several females (Madison, 1980). Under these conditions, males' fitness is maximized by mating rather than parenting effort (Clutton-Brock, 1991), whereas females may increase fitness by selective mate choice after intermale competition before mating or after conception (Storey, 1994).

However, during the colder months of the year, meadow voles, like many rodents, live communally to conserve heat (Howard, 1950). During autumn, social nesting and breeding activity overlap in time, and reproductive males have been observed nesting with females and preweanling young (Madison, FitzGerald, & McShea, 1984). In autumn in some years, up to 50% of females produce litters (Christian, 1980; Tamarin, 1977), and in unusually mild winters, 100% of females may continue breeding (Webster & Brooks, 1981). During fall, meadow vole population densities are lower than in the primary, summer breeding season, and offspring mortality is likely greater. Under these marginal free-living conditions, the facultative initiation of pair bonding and biparental care may confer a selective advantage for males by affording postpartum mating opportunities with a known breeding female and greater offspring survivorship. For females, the presence of the sire likely reduces the energetic demands characterized by uniparental care: sole guarding of pups from aggressive conspecifics and elevated metabolic activity to ensure adequate thermoregulation of the litter.

In monogamous *Microtus ochrogaster* (prairie voles), a partner preference is defined as spending twice as much time in contact with a familiar partner relative to an unfamiliar conspecific (Insel, Preston, & Winslow, 1995; Williams, Catania, & Carter, 1992). Previous laboratory research has shown that after 24 hr of cohabitation with a male, meadow vole females spend 55% of a 3-hr preference test in lateral contact with a familiar partner, versus 6% spent with an unfamiliar male (Parker, Phillips, & Lee, 2001). These data represent greater than a twofold difference between time spent with a partner versus stranger and lend support to the theory that characteristically nonmonogamous female meadow voles may form selective preferences for opposite-sex partners under specific circumstances.

The neurobiology underlying the development of affiliative behaviors has been examined (Carter, Lederhendler, & Kirkpatrick, 1997). Oxytocin (OT), a nine-amino-acid peptide hormone with diverse neural forebrain projections, has been implicated in regulating the development of pair bonds in female prairie voles (Witt, Carter, & Walton, 1990). Specifically, central infusion of OT agonists and antagonists induce or inhibit, respectively, the

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This research was supported by National Institutes of Health Reproductive Sciences Program Training Grant 5T32HD07048 to Karen J. Parker and by National Institute of Child Health and Human Development Grant HD24575 and National Science Foundation Grant IBN9808814 to Theresa M. Lee. We thank Tammy Jechura for expert computer assistance, Israel Liberzon for the receptor binding protocol, and Bob Thompson for generous use of laboratory facilities and equipment.

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development of female partner preferences (Insel & Hulihan, 1995).

Although the relationship between OT receptor number and distribution and onset of partner preferences has not been examined in any species, comparative data from sexually inexperienced (hereafter naive), monogamous prairie voles and naive, nonmonogamous *Microtus montanus* (montane voles) are available (Insel & Shapiro, 1992). Findings from this study suggest that differences in OT receptor distribution in the extended amygdala neural pathway (e.g., the accessory olfactory nucleus [AON], lateral septum [LS], bed nucleus of the stria terminalis [BNST], and amygdala) are associated with differences in affiliative behavior and social organization. In this study, OT receptor distribution was also assessed in naive LD meadow vole females and naive, socially monogamous female pine voles (Insel & Shapiro, 1992). Interestingly, unlike the stark regional receptor differences observed in prairie and montane vole females, meadow and pine vole females only showed regional receptor differences in the LS but did not significantly differ in OT binding in the BNST, amygdala, or other areas associated with affiliative behavior (Insel et al., 1993; Witt, 1995, 1997). These data suggest that the "intermediate" binding pattern found in meadow and pine voles (compared with that of prairie and montane voles) may reflect the ability of meadow and pine voles to change their social behaviors facultatively to best suit socioecological conditions. This is supported by field evidence that intraspecific variation in free-living meadow and pine vole social organization is commonly observed (Fitzgerald & Madison, 1983; Madison et al., 1984).

Meadow vole research in our laboratory provides an ideal model by which to examine whether OT receptor patterns are associated with affiliative behavior, because the circumstances under which female meadow voles behave socially (Parker & Lee, 1998) and develop selective partner preferences have been previously identified (Parker et al., 2001). In this previous experiment, social behavior and partner preferences were assessed using a choice apparatus in which the test vole chose to spend time with a familiar partner or stranger. Females were housed under either summer, long-day (LD) lengths (14 hr light/day), when free-living females are territorial, or winter, short day (SD) lengths (10 hr light/day), when free-living females live socially, and tested (within photoperiod) under six different conditions after no social contact with a male (e.g., naive); 24 hr, 10 days, or 23 days of mated cohabitation with a male; or 24 hr or 10 days of unmated cohabitation with a male. It was initially hypothesized that (a) naive females housed under SD would engage in more social contact with unknown voles than LD females and (b) after social manipulations, SD females would more readily form selective partner preferences, both presumably because of seasonal differences in free-living socioecology. The original predictions were partially supported; with no social cohabitation, naive SD females spent more time in contact with unknown voles than their LD counterparts. However, both LD and SD females formed partner preferences after 24 hr of mated cohabitation with a male, and neither longer cohabitation periods nor delivery of the litter (i.e., 23 d) increased this preference. However, when females were housed under SD, but not LD, conditions they failed to form partner preferences after only 24 hr of unmated cohabitation. Nevertheless, after 10 days of unmated cohabitation, SD female partner preferences were identical to those established after 24 hr of mated cohabitation (in both LD and SD conditions) and 24 hr of unmated cohabitation (in LD conditions).

Because meadow voles demonstrate seasonal changes in social organization that are reflected by social capacity in the naive state and also develop selective partner preferences that are equivalent to those of monogamous prairie voles (Williams et al., 1992), the goals of the present experiment were to determine (a) whether social, naive SD females showed greater regional OT receptor binding than asocial, naive LD females, (b) whether the presence or absence of selective partner preferences was associated with regional OT receptor binding, and (c) whether partner preferences were differentially associated with regional OT receptor binding under SD and LD conditions.

Method

Subjects

Subjects, derived from wild-caught meadow voles (*Microtus pennsylvanicus*) indigenous to northwestern Pennsylvania and southwestern New York State, were born to breeding pairs in an established colony at the University of Michigan. Weanling meadow vole pups were removed from the dam at 19 days of age and housed in same-sex sibling dyads in LD (14 hr light/day) or SD (10 hr light/day) conditions. Subjects (N = 39 for autoradiography) were housed in 26.67 × 21.59 × 13.97-cm polypropylene cages on pine shaving bedding with food (Purina Mouse Chow no. 5015, Ralston-Purina, St. Louis, MO) and water available *ad libitum*. Vole rooms were maintained at 21 ± 2° C with low ambient noise conditions. Subjects remained so housed until the experimental procedure commenced (11–13 weeks of age).

Partner Preference Testing

Partner preference testing (described later; see also Parker et al., 2001) occurred either within 24 to 36 hr after mating or, for the females that did not mate, 24 to 36 hr after pairing. Other females were tested for partner preferences after 10 to 11 days of mated or unmated cohabitation. Finally, the preferences of mated females were tested 48 to 72 hr after the birth of the litter (after 23 to 24 days of cohabitation). Control females were tested once at comparable ages to study voles at the first postmating test.

Behavioral testing was conducted in a Plexiglas affiliation device (39.37 × 24.13 × 27.94 cm). The device consisted of three equal compartments connected by a runway on one side. In one of the compartments, the male from the test pair was loosely tethered to the back wall of the compartment. The familiar male was able to move freely in his end of the compartment. A second unfamiliar male was tethered in a second arm of the device. The third arm of the device remained empty. Tethered males were allowed to become familiar with the new environment for 10 min before the female was introduced into the device. During the 3-hr preference test, the female could move freely throughout the entire testing device. Behavioral tests were scored with a computer program for counts and durations of amicable and agonistic behavior between the test and stimulus voles. Behavior was determined to be amicable when the test vole engaged in side by side contact with a stimulus vole (see Williams et al., 1992). Behavior was determined to be aggressive when the test vole engaged in charging, attacking, biting, or boxing with a stimulus vole (see Ferkin, 1988).

Receptor Autoradiography

After partner preference testing, voles were anesthetized with halothane and decapitated. Brains were quickly removed, frozen on dry ice, and stored at −70° C until sectioned. On the basis of statistical analyses of

partner preferences, several LD and SD female groups were selected for central OT receptor autoradiography. Because LD females manifest partner preferences after just 24 hr of social cohabitation (and mating and longer cohabitation periods do not enhance this effect), the following groups were selected for examination: LD females after no social cohabitation (e.g., naive condition; $n = 5$) or after 24 hr of unmated cohabitation ($n = 5$). SD females formed partner preferences under slightly different circumstances. Consequently, more groups were selected for examination: after no social cohabitation (e.g., naive condition; $n = 7$), after 24 hr of unmated cohabitation (when SD females did not exhibit preferences; $n = 5$), after 24 hr of mated cohabitation ($n = 5$), after 10 days of unmated cohabitation ($n = 5$), and 3 days postpartum ($n = 7$). Females of the last three groups displayed preferences.

Brain slices of 20 μm were cut in a cryostat at 19 ± 1° C, slide mounted (Colorfrost/Plus slides, Fisher Scientific, Santa Clara, CA), and stored at −70°C. Coronal sections were cut through four brain areas from rostral to caudal. Brain slices were thawed for 30 min at room temperature before commencement of the binding assay. OT receptor autoradiography was performed using a highly selective [^{125}I]-ornithine vasotocin analog, d(CH$_2$)$_5$[Tyr(Me)^2, Thr^4,Orn^8,[^{125}I] Tyr^9-NH$_2$] (New England Nuclear, Wellesley, MA; 2,200 Ci/mmol, 0.1 nM). Slides were incubated in buffer (50 mmol Tris, 10 mM magnesium chloride, 0.1% bovine serum albumin [BSA]) for 60 min, washed three times (8 min each) in buffer (50 mmol Tris, 100 mmol choline chloride, 0.1% BSA, 0.01% Triton X-100), and briefly dipped in double-distilled water. All buffer chemicals were obtained from Sigma Chemical (St. Louis, MO). Nonspecific binding was determined using 1-μmol concentration of OT, Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH$_2$ (Peninsula Laboratories, Belmont, CA). Slides were dried under a stream of cool air, placed in film cassettes, and apposed to BioMax MR X-ray film (Amersham, Buckinghamshire, England) for 24 hr. In preliminary trials, x-ray films were developed after different lengths of exposure (e.g., 24 hr, 48 hr, and 72 hr) to determine a suitable exposure time that was in the linear response range of the film. Film optical densities were measured by an experimenter (who was unaware of experimental conditions) using Image J for the PC, a computerized imaging system from the National Institutes of Health.

Neuroanatomical Measurements and Statistics

For each subject, nine sections per vole per area per side of brain were analyzed (i.e., six specific binding sections and three nonspecific binding section per vole per area per side of the brain). In a few cases, tissue sections were damaged during autoradiographic processing and were excluded from analysis by the mutual consent of two experimenters, both of whom were unaware of subjects' experimental condition. Specific binding and nonspecific binding were averaged separately for each side of the brain, nonspecific binding was subtracted from specific binding for each side of the brain to yield total binding for left and right structural areas, and finally, left and right sides were averaged together to represent a single data point for each vole/area. Brain regions for image analysis were selected according to previous work that implicated a possible role for the AON, LS, BNST, LatAmyg, and central amygdala (CenAmyg) in mediating affiliative and maternal behaviors in various vole and rat species (Insel, 1986; Insel & Shapiro, 1992). These brain areas are part of the extended amygdala neural pathway (AON-amygdala-BNST-septum-medial preoptic area), which expresses increased neural activation after social/sexual/parental interactions (reviewed by Newman, 1999).

To examine whether behavioral state was associated with OT receptor distribution, two-group t tests (when two groups were compared) or a one-way analysis of variance (ANOVA) with affiliative state as the primary factor (when more than two groups were compared) assessed differences in OT receptor binding for each neuroanatomical area. For ANOVA, when significant main effect differences in OT binding were observed, post hoc pairwise comparisons were used to test for group differences ($p < .05$).

Results

Effect of Photoperiod on Affiliative Behavior and OT Receptor Density in Naive Voles

In the affiliation test, naive SD females spent significantly more time in contact with unknown stimulus voles, $t(9) = 2.681$, $p = .025$, compared with their LD counterparts. SD females had higher OT receptor binding in the lateral septum, $t(10) = 2.179$, $p = .054$; lateral amygdala, $t(10) = 2.995$, $p = .013$; and central amygdala, $t(10) = 2.832$, $p = .018$, compared with LD females. However, SD and LD naive females did not differ in OT binding in the AON, $t(5) = 1.042$, $p = .345$; or BNST, $t(9) = 1.337$, $p = .214$ (see Figures 1, 2, and 3).

Effect of 24 Hr of Unmated Cohabitation on OT Receptor Density in LD Females

Twenty-four hours of unmated cohabitation with an opposite-sex partner is sufficient to induce selective partner preferences in LD females (Parker et al., 2001). Data analysis revealed that partner preference onset occurs concomitantly with greater OT receptor binding in the AON, $t(6) = -4.303$, $p = .005$; LS, $t(8) = -2.905$, $p = .020$; and BNST, $t(7) = -2.661$, $p = .032$, compared with naive LD females. A similar trend was observed for the lateral amygdala, $t(8) = -1.871$, $p = .098$; and the central amygdala, $t(8) = -1.978$, $p = .083$, although neither finding was significant (possibly because of sample size and conservative two-tailed tests;

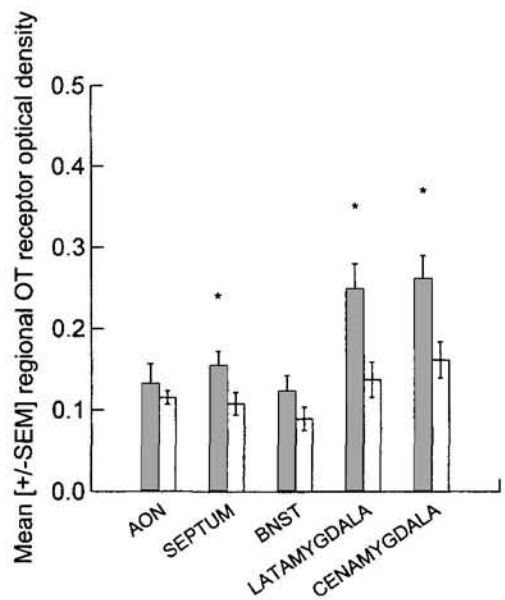

Figure 1. Regional central oxytocin (OT) receptor optical density is associated with greater sociality in sexually inexperienced (naive) short day (SD, solid bars), but not long day (LD, open bars), females. SD females have greater OT binding in the lateral septum (SEPTUM), lateral amygdala (LATAMYGDALA), and central amygdala (CENAMYGDALA) than LD females, but SD and LD females do not differ in the anterior olfactory nucleus (AON) or bed nucleus of the stria terminalis (BNST). Asterisks indicate a minimum significant difference ($p < .05$) between LD and SD females in a specific neuroanatomical area.

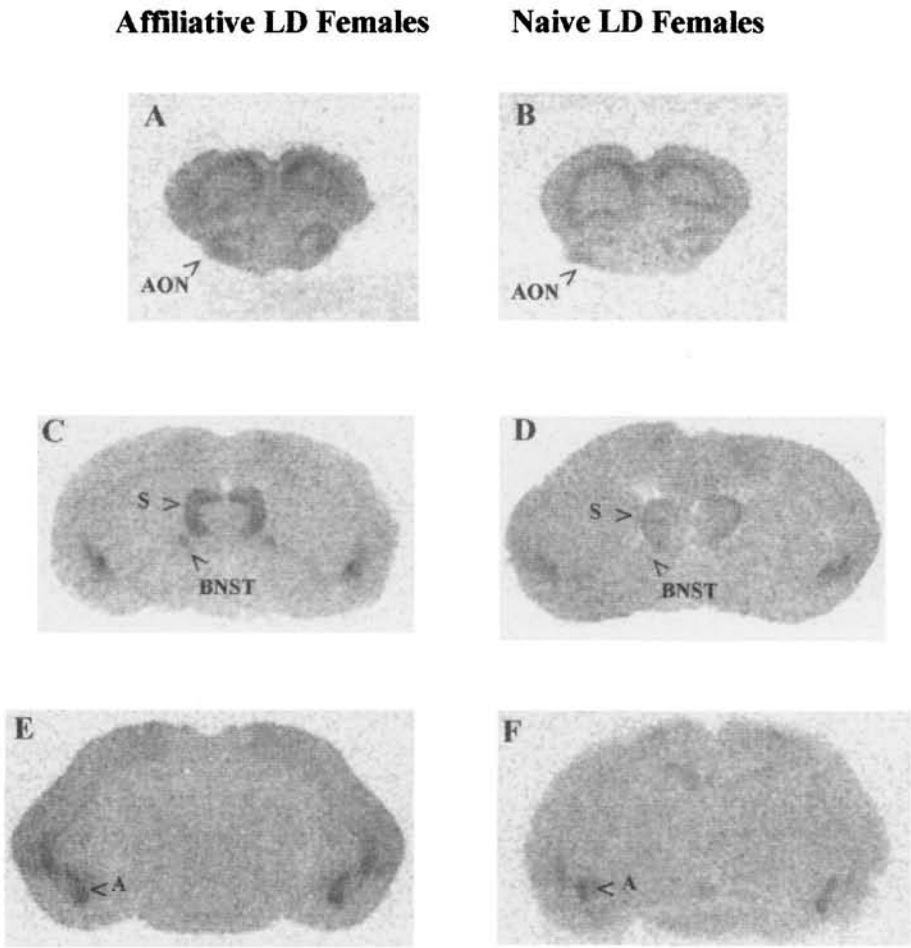

Figure 2. Photomicrographs of oxytocin receptor optical densities of long day (LD) female extended amygdala structures after either 24 hr of unmated cohabitation (affiliative) or no social cohabitation (naive). A and B: anterior olfactory nucleus (AON); C and D: lateral septum (S) and bed nucleus of the stria terminalis (BNST) at the level of the anterior commissure; E and F: lateral and central amygdala (A).

see Figures 2 and 4). Interestingly, LD females, after 24 hr of cohabitation, have equivalent OT receptor binding to SD naive females in each neuroanatomical area (see Figures 1, 2, 3, and 4).

Effect of Unmated Cohabitation Duration on OT Receptor Density in SD Females

In the absence of social cohabitation, naive SD females maintain high OT receptor binding in the extended amygdala compared with LD voles. After 24 hr of unmated cohabitation with a male, SD females fail to develop partner preferences, but, during 10 days of unmated cohabitation, form strong partner preferences for an opposite-sex partner (Parker et al., 2001). When these three groups were compared for regional OT receptor binding, differences were observed in both the LatAmyg, $F(2, 14) = 3.662$, $p = .053$; and CenAmyg, $F(2, 14) = 4.422$, $p = .032$. In particular, after 24 hr of unmated cohabitation, females showed less OT receptor binding in the LatAmyg than females that lived with a male for 10 days ($p = .022$) and those that never lived with a male ($p = .057$). The latter two groups did not differ from each other. Similar findings were observed for the CenAmyg; unmated females that lived with a male for 24 hr had less CenAmyg OT receptor binding than either unmated females that lived with a male for 10 days ($p = .027$) or females that never lived with a male ($p = .016$). The latter two groups did not differ from each other. No overall differences were observed for regional OT binding in the AON, $F(2, 8) = 1.190$, $p = .353$; LS, $F(2, 14) = 2.076$, $p = .162$; or BNST, $F(2, 14) = 0.418$, $p = .667$ (see Figures 3, 5, and 6).

Effect of Mated Cohabitation Duration on OT Receptor Density in SD Females

Like LD females, SD females form strong partner preferences after 24 hr of mated cohabitation with a male. Longer cohabitation periods (e.g., 10 days, 23 days) do not enhance partner preferences, but after 23 days of mated cohabitation (2 days after delivery of the litter; hereafter postpartum females), postpartum females exhibit robust maternal behavior (Reeves, 1994) and first initiate stranger-

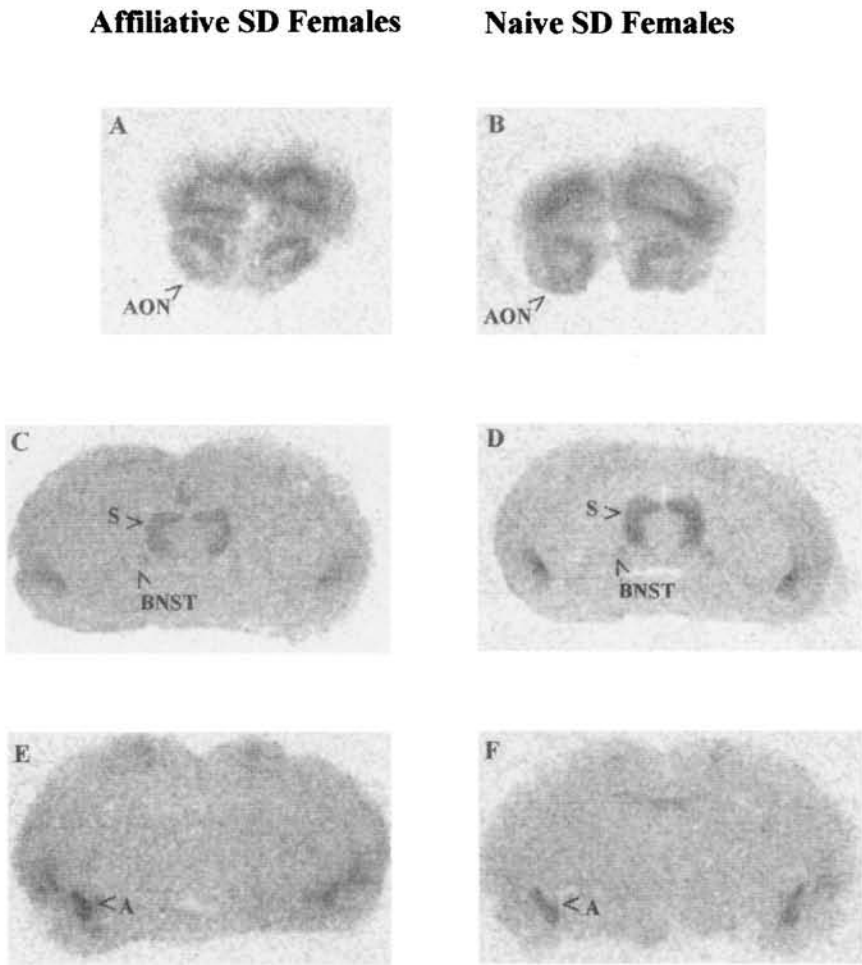

Figure 3. Photomicrographs of oxytocin receptor optical densities of short day (SD) female extended amygdala structures after either 24 hr of mated cohabitation (affiliative) or no mated cohabitation (naive). A and B: anterior olfactory nucleus (AON); C and D: lateral septum (S) and bed nucleus of the stria terminalis (BNST) at the level of the anterior commissure; E and F: lateral and central amygdala (A).

directed aggression (Parker et al., 2001). Thus, whether OT binding differed between the three groups that showed behavioral differences was examined after no social cohabitation (i.e., naive females), after 24 hr of mated cohabitation, and after 23 days of mated cohabitation (i.e., postpartum females). When these three groups were compared for differences in regional OT receptor binding, only one overall difference was observed. These groups significantly differed in OT binding in the AON, $F(2, 8) = 8.882$, $p = .009$, such that postpartum females showed less OT binding than either naive females ($p = .036$) or females after 24 hr of mated cohabitation ($p = .003$). These latter two groups did not differ from each other. No differences were observed for the LS, $F(2, 15) = 1.002$, $p = .391$; BNST, $F(2, 15) = 0.130$, $p = .879$; LatAmyg, $F(2, 15) = 0.480$, $p = .628$; or CenAmyg, $F(2, 15) = 0.692$, $p = .516$ (see Figures 3 and 7).

Discussion

The first goal of this experiment was to determine whether seasonal changes in social organization were associated with OT receptor binding density and distribution in the extended amygdala in female meadow voles. Naive SD females showed significantly higher OT receptor binding in the LS, LatAmyg, and CenAmyg compared with naive LD females. The relationship between OT receptor density and affiliative behavior is supported by a previous study that demonstrated an association between OT receptor patterns and interspecific differences in social organization (Insel & Shapiro, 1992). It is also consistent with meadow vole field and laboratory data that show that SD females live socially during colder (Madison et al., 1984) but not warmer (Madison, 1980) months, and that naive SD females engage in more lateral contact with unknown males during behavioral testing than naive LD females (this study).

The second goal of this experiment was to examine whether OT receptor binding density and distribution, within photoperiod, differed between females that exhibited partner preferences and those that did not. Previous research in our laboratory has shown that LD females acquire selective partner preferences after 24 hr of social cohabitation with a male compared with naive LD animals (Parker

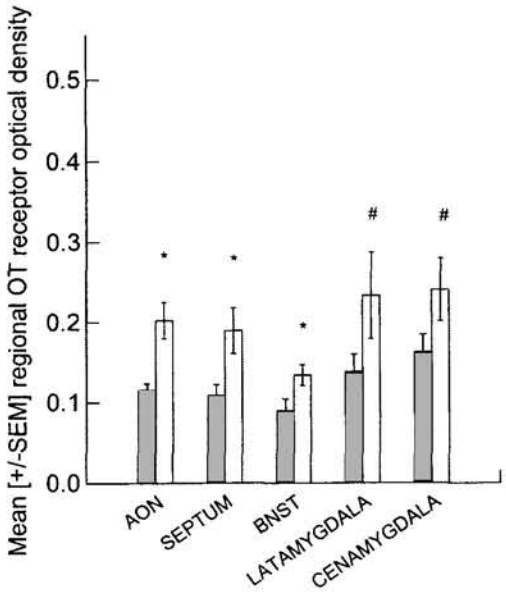

Figure 4. After 24 hr of unmated cohabitation (open bars) with a male, long day (LD) females develop selective partner preferences, and this is associated with greater oxytocin (OT) receptor binding in extended amygdala structures compared with naive (solid bars) LD females. Asterisks ($p < .05$) and pound symbols ($p < .10$) indicate a difference between LD females in a specific neuroanatomical area. AON = anterior olfactory nucleus; SEPTUM = lateral septum; BNST = bed nucleus of the stria terminalis; LATAMYGDALA = lateral amygdala; CENAMYGDALA = central amygdala.

et al., 2001). Data from this experiment established that greater OT receptor binding in the AON, LS, and BNST and several near-significant differences in the amygdala were found for LD females that exhibited partner preferences and those that did not (i.e., naive LD females). For SD females, which demonstrate appreciable OT receptor binding in the extended amygdala as naive voles, no differences in regional OT binding were found after the development of selective partner preferences after 24 hr of mated cohabitation or 10 days of unmated cohabitation with a male. Nonetheless, several differences in central OT receptor binding occurred with differences in SD behavior. After 24 hr of unmated cohabitation, SD females failed to develop partner preferences and had lower OT receptor binding in the LatAmyg and CenAmyg compared with both naive SD females and SD females that formed partner preferences after longer cohabitation periods. Lower OT receptor binding was also observed for postpartum SD females in the AON compared with naive females and those exhibiting partner preferences. (Because maternal behavior has never been assessed in naive, cohabiting, or pregnant SD meadow vole females, a functional role cannot be posited as to whether lower OT receptor density in the AON of postpartum females is associated with maternal behavior onset.) Taken together, these data are the first to show that central OT receptor binding and distribution are associated with seasonal changes in intraspecific social organization and the development of selective partner preferences in a nonmonogamous microtine rodent.

Whether or not activity in meadow vole OT neural pathways functions to generate the greater sociality observed in naive SD females and the development of partner preference formation in both LD and SD females remains unknown and awaits further investigation by other experimental techniques. Nonetheless, findings from experiments in other rodent species suggest several intriguing possibilities for the functional significance of these observed OT receptor patterns in relation to meadow vole social organization and affiliative state.

In the present studies, greater sociality in naive SD females occurred concomitantly with higher OT receptor binding in the lateral septum, LatAmyg, and CenAmyg. In many species, lesions of the lateral septum and various amygdaloid nuclei increase species-specific agonistic behavior (reviewed by Albert & Walsh, 1984), implicating an inhibitory role for these structures in regulating conspecific aggression. Additionally, lesions of the septum disrupt maternal behavior (mice, Carlson & Thomas, 1968; rats, Fleischer & Slotnick, 1978), and in montane voles greater OT receptor binding in the LatAmyg is associated with the onset of maternal behavior (Insel & Shapiro, 1992). Because the location of OT receptor sites demarcates where endogenous peptides can exert

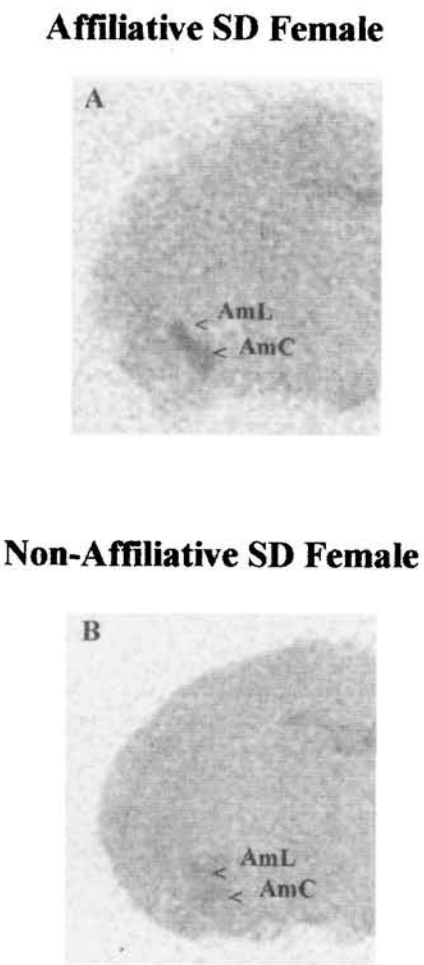

Figure 5. Photomicrographs of oxytocin receptor optical densities of short day (SD) female lateral (AmL) and central (AmC) amygdaloid nuclei after either (A) 24 hr of mated cohabitation (affiliative) or (B) 24 hr of unmated cohabitation (nonaffiliative).

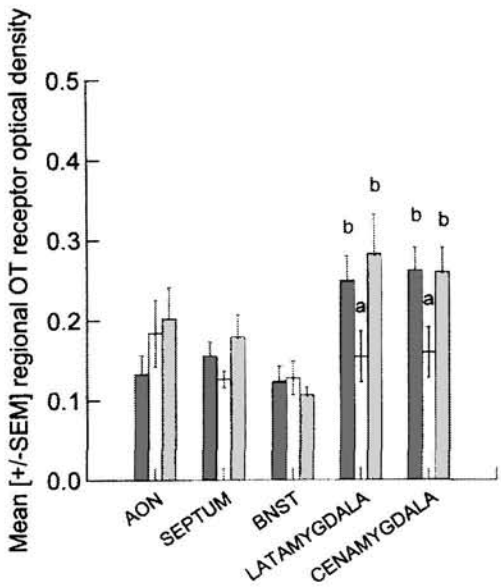

Figure 6. In short day (SD) females, failure to develop partner preferences after 24 hr of unmated cohabitation (open bars) with a male is marked by lower oxytocin (OT) receptor binding in the lateral amygdala (LATAMYGDALA) and central amygdala (CENAMYGDALA) compared with naive SD females (dark gray bars) and SD females that cohabited with a male for 10 days (light gray bars). a indicates a minimum significant difference ($p < .05$) between groups; b indicates no significant difference between groups. AON = anterior olfactory nucleus; SEPTUM = lateral septum; BNST = bed nucleus of the stria terminalis.

their effects, perhaps increased OT activity in these specific areas inhibits object-appropriate aggression, which is a necessary prerequisite for the expression of affiliative and maternal behaviors. Thus, the comparatively greater OT receptor binding in the extended amygdala of meadow voles may reflect free-living circumstances, which typically facilitate greater sociality in communally living SD, but not territorially living LD, females.

Because meadow voles exhibit these seasonal differences in social organization, we previously hypothesized that SD females would develop selective partner preferences more rapidly than LD females, as selective affiliation in overwintering breeding pairs might serve to offset fitness costs associated with the harsh conditions that characterize SD, but rarely LD, conditions (Parker et al., 2001). Nonetheless, it was also predicted that LD females might develop partner preferences, albeit at a slower rate, because under low-density summer breeding populations mates are scarce, and this occurs on a regular, cyclical basis for meadow voles (Christian, 1980; Taitt & Krebs, 1985). Although SD and LD females develop partner preferences rapidly and with equal facility, only LD females show differences in OT receptor binding, which occur concomitantly with the development of partner preferences. In contrast, social SD meadow voles are more like prairie voles, because neither meadow voles nor presumably prairie voles (Insel & Shapiro, 1992) demonstrate differences in OT receptor binding in conjunction with the onset of affiliative preferences. Because LD females may only live with males rarely and opportunistically, whereas SD females, like prairie voles, frequently live in field nests with an adult male and preweanling young (Madison et al., 1984), these differences in OT receptor binding may reflect the "facultative" nature of LD and potentially more consistent nature of SD, selective partner preference formation.

The reason why SD females fail to form partner preferences after 24 hr of unmated cohabitation is unknown. One possible interpretation of these data is that, because meadow vole reproductive activity decreases during winter months, reproductive physiology is frequently suppressed by winter conditions (Meek & Lee, 1993). Because this translates into a reduction in gonadal steroids that facilitate partner preference formation in prairie voles (Williams et al., 1992; Witt, 1997), perhaps SD females experience a greater latency to activation of partner preferences. However, exactly how estrogen interacts with OT neural activity and whether either of these is involved in the formation of meadow vole partner preferences remain to be investigated. Nonetheless, the finding that these females show less OT receptor binding in the LatAmyg and CenAmyg than unmated females, which do show partner preferences after longer cohabitation periods, suggests that the amygdala may be an important neuroanatomical target for central OT during the development of selective partner preferences.

In conclusion, it was demonstrated that (a) central OT receptor binding and distribution are associated with seasonal changes in intraspecific social organization in meadow voles housed under LD and SD lengths, (b) differences in OT receptor binding are associated with affiliative state in a nonmonogamous rodent, and

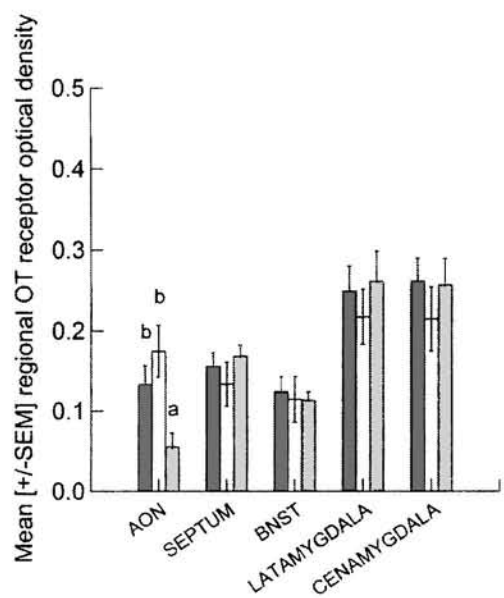

Figure 7. Partner preference onset in mated short day (SD) females, compared with naive SD females (dark gray bars), is not associated with appreciable differences in regional oxytocin (OT) receptor binding as in long day females (see Figures 2 and 4). Only one significant difference in OT receptor binding, in the extended amygdala (i.e., anterior olfactory nucleus [AON]), is associated with 23 days of mated cohabitation (light gray bars) and delivery of the litter. Open bars represent females in the 24-hr cohabitation group. a indicates a minimum significant difference ($p < .05$) between groups; b indicates no significant difference between groups. SEPTUM = lateral septum; BNST = bed nucleus of the stria terminalis; LATAMYGDALA = lateral amygdala; CENAMYGDALA = central amygdala.

(c) the presence of partner preferences is associated with differences in OT receptors (compared with the naive state) in LD but not SD female meadow voles. Taken together, these photoperiodic differences in regional OT receptor binding may reflect differences in life history strategies in free-living meadow vole populations during the summer and fall breeding seasons.

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Received November 6, 2000
Revision received April 13, 2001
Accepted July 10, 2001 ■