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Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles

(microtine/affiliation/parental behavior/amygdala/septum)

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ABSTRACT **The neuropeptide oxytocin has been implicated in the mediation of several forms of affiliative behavior including parental care, grooming, and sex behavior. Here we demonstrate that species from the genus *Microtus* (voles) selected for differences in social affiliation show contrasting patterns of oxytocin receptor expression in brain. By *in vitro* receptor autoradiography with an iodinated oxytocin analogue, specific binding to brain oxytocin receptors was observed in both the monogamous prairie vole (*Microtus ochrogaster*) and the polygamous montane vole (*Microtus montanus*). In the prairie vole, oxytocin receptor density was highest in the prelimbic cortex, bed nucleus of the stria terminalis, nucleus accumbens, midline nuclei of the thalamus, and the lateral aspects of the amygdala. These brain areas showed little binding in the montane vole, in which oxytocin receptors were localized to the lateral septum, ventromedial nucleus of the hypothalamus, and cortical nucleus of the amygdala. Similar differences in brain oxytocin receptor distribution were observed in two additional species, the monogamous pine vole (*Microtus pinetorum*) and the polygamous meadow vole (*Microtus pennsylvanicus*). Receptor distributions for two other neurotransmitter systems implicated in the mediation of social behavior, benzodiazepines, and μ opioids did not show comparable species differences. Furthermore, in the montane vole, which shows little affiliative behavior except during the postpartum period, brain oxytocin receptor distribution changed within 24 hr of parturition, concurrent with the onset of maternal behavior. We suggest that variable expression of the oxytocin receptor in brain may be an important mechanism in evolution of species-typical differences in social bonding and affiliative behavior.**

Social organization and mating systems display a remarkable degree of diversity. In an effort to explain this behavioral variability, ethologists have compared large groups of species to discern correlations between social organization and ecology (1–3). This comparative research strategy has significantly increased our understanding of the selective pressures responsible for the evolution of much of this behavioral diversity. Curiously, however, the neural mechanisms underlying such differences in behavior have received little systematic study and remain poorly understood. In the present paper, we use a comparative approach to examine differences in neurotransmitter receptor maps in closely related species that display marked differences in social and reproductive behavior.

Voles of the genus *Microtus* display a wide array of social and mating systems. For instance, prairie voles (*Microtus ochrogaster*) and montane voles (*Microtus montanus*), two species that are similar in appearance and in many aspects of nonsocial behavior (4), display marked differences in social organization. In their natural habitat, prairie voles frequently form long-term monogamous relationships and show high levels of parental care (5–7). Even in the laboratory, adult prairie voles usually sit side-by-side with a mate and attack unfamiliar adults; both sexes display parental care (8–10). In contrast, montane vole adults live in isolated burrows and show no evidence of monogamy (11). In the laboratory, montane voles appear minimally parental and generally spend little time in contact with conspecifics (10, 12). Species differences in social behavior are evident very early in life, as prairie vole young show marked increases in ultrasonic calls and glucocorticoid secretion in response to social isolation, whereas montane vole pups show little if any behavioral or physiologic response to separation from the nest (13). This species difference in the newborn pup's response to separation suggests that an inherent difference in the propensity for social contact or affiliation underlies the differences in social organization evident throughout the life-span. The comparative analyses of these species thus provide an excellent model system with which to study the neuroethology of complex social behavior.

The neurochemical determinants of social affiliation remain unknown, although several neurotransmitter systems appear to affect various aspects of attachment behavior. Endogenous opioids have been implicated in the mediation of grooming (14) as well as in the alleviation of separation distress (15). The benzodiazepine–γ-aminobutyric acid receptor complex appears to mediate aspects of both maternal behavior (16) and infant separation distress (17). Recently, various studies have suggested that oxytocin (OT) may play an important role in central mediation of several forms of affiliation (reviewed in ref. 18). In this study, we initially analyzed receptor distributions for each of these neurotransmitters in prairie and montane voles. To extend these initial comparisons further, we subsequently examined neural distributions of receptors in two additional species from the same genus, the monogamous pine vole (*Microtus pinetorum*) and the polygamous meadow vole (*Microtus pennsylvanicus*).

MATERIALS AND METHODS

Animals. Both male and female prairie and montane voles from our own breeding colonies were studied. Pine and meadow voles were obtained from D. A. Dewsbury (University of Florida, Gainesville). All animals were weaned at 21 days of age and housed with littermates of the same sex until 45–90 days of age, when they were used for autoradiographic experiments. Housing was on a 14:10-hr light/dark schedule with food and water available ad libitum.

Receptor Autoradiography. Brains were quickly removed, frozen on dry ice, and stored at −70°C until sectioned. Cryostat-cut 20-μm slide-mounted sections were used for the receptor binding assay. Adjacent sections were used for analysis of binding to OT, μ-opioid, and benzodiazepine

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Abbreviations: OT, oxytocin; OTA, oxytocin antagonist; DAGO, [D-Ala^2,*N*-Me-Phe^4,Gly^7-Ol]eukephalin; BNST, bed nucleus of the stria terminalis; AVP, [Arg^8]vasopressin.

receptors. The specific protocols for receptor binding have been published (19–21). For assay of OT receptors, the ligand was ^{125}I-labeled d(CH$_2$)$_5$-[Tyr(Me)^2,Thr^4,Tyr-NH$_2^9$]ornithine vasotocin (^{125}I-OTA; specific activity, 2200 Ci/mmol; 1 Ci = 37 GBq), a selective, high-affinity receptor antagonist (21) with unlabeled 0.5 μM OT added to the incubation buffer to define nonspecific binding in adjacent sections. For μ-opioid receptors, the ligand was [^{3}H][D-Ala^2,*N*-Me-Phe^4,Gly^7-ol]enkephalin ([^{3}H]DAGO) at a concentration of 1 nM (specific activity, 46.8 Ci/mmol). Nonspecific binding was defined by addition of unlabeled naloxone (1 μM). For benzodiazepine receptors, the ligand was [^{3}H]flunitrazepam (1 nM; specific activity, 81.9 Ci/mmol), and nonspecific binding was defined with diazepam (1 μM).

When dry, slides were apposed to Hyperfilm (Amersham) along with ^{125}I plastic standards for 2–3 days (^{125}I-OTA) or ^{3}H plastic standards for 3 ([^{3}H]flunitrazepam) or 8 ([^{3}H]DAGO) weeks. The resulting autoradiograms were analyzed with the IMAGE program for the Macintosh, permitting conversion of optical density to fmol per mg of protein equivalents by using a third-order polynomial regression. Nonspecific binding was subtracted from total binding to yield values for specific binding. Anatomic localization was defined by counterstaining postfixed sections with thionin and overlaying computer images of stained sections and autoradiograms. All radionuclides were obtained from DuPont/NEN; standards were obtained from Amersham.

Homogenate Binding. The kinetics and specificity of ^{125}I-OTA binding were analyzed in homogenates of 600-μm sections through the septum and anterior hypothalamus. Tissue was homogenized (10 sec on polytron setting 6) in 50 mM Tris buffer (pH 7.4), spun at 40,000 × *g*, and resuspended in incubation buffer. Tissue (100 μl), tracer (50 pM; 100 μl), and either buffer (100 μl) or various concentrations of displacer were incubated for 90 min at room temperature before separation over filters (GFB; Whatman) that had been presoaked for 1 hr in 1% bovine serum albumin. Filters were washed three times in 5 ml of ice-cold Tris buffer and then counted with a γ counter (efficiency, 80%).

Behavior Studies. Lactating females were tested within 24 hr of parturition. Behavioral testing used a two-compartment Plexiglas Y-maze apparatus consisting of two 53-cm-long, 12-cm-wide, and 12-cm-high arms. An animal was placed in the start box and two congeneric pups (2–8 days old) were placed at the end of one arm of the Y-maze. Parental behavior was recorded as time spent with pups either nursing, grooming, or crouching during a 5-min period. Females were decapitated the same day for receptor binding studies.

Data Analysis. For autoradiographic studies, all slides from a given region were processed simultaneously with each run including at least three males and three females of each species. Analysis by region included a two-way ANOVA with main factors of species and gender. Differences at the $P = 0.05$ level were considered significant.

RESULTS

Receptor Distribution. Because we were primarily interested in species differences in limbic structures, we examined receptor distribution across the forebrain. Specific binding of ^{125}I-OTA was found in several regions in both species, but the areas of greatest binding were generally nonoverlapping (Fig. 1). As shown in Table 1, prairie voles had severalfold higher levels of ^{125}I-OTA binding in the nucleus accumbens, prelimbic cortex, bed nucleus of the stria terminalis (BNST), lateral amygdala, and midline thalamic nuclei. Montane voles had higher levels of ^{125}I-OTA binding in the lateral septum, ventromedial nucleus, and cortical nucleus of the amygdala. There were no sex differences in ^{125}I-OTA binding in either species.

In two further studies, these species differences in binding appeared as differences in receptor number and not differences in affinity. Under saturating conditions (400 pM ^{125}I-OTA), binding in the lateral septum of the montane vole was 2.6 times that of the prairie vole ($t_{(6)} = 7.25$; $P < 0.001$), approximating the 2.7-fold difference observed at the 40 pM concentration (Table 1). Furthermore, in homogenate binding studies, the affinity (K_d) for binding of ^{125}I-OTA was virtually identical in the two species (Fig. 2). Competition studies further demonstrated that in both species ^{125}I-OTA was binding to an OT receptor rather than to an [Arg^8]vasopressin (AVP) receptor (Fig. 2). This pharmacologic characterization resembles several previous reports of binding to OT receptors in rat brain (22–24).

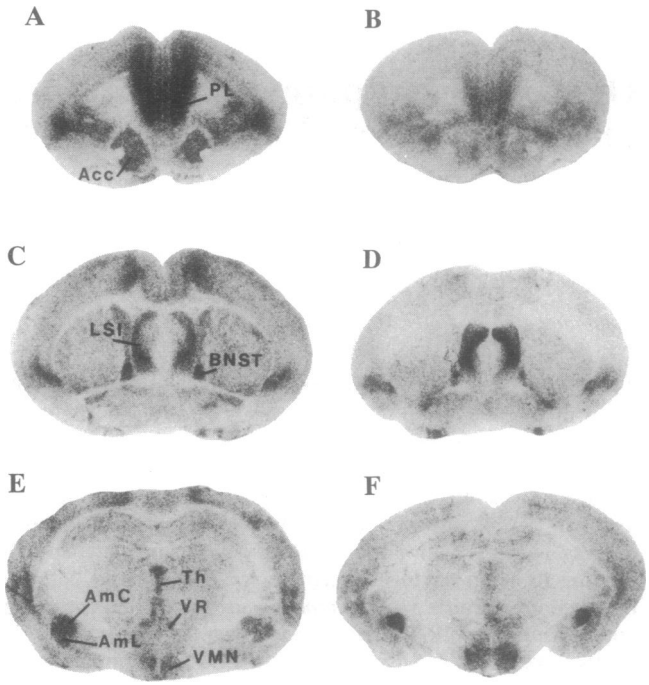

FIG. 1. Representative autoradiograms of ^{125}I-OTA binding (40 pM) to 20-μm coronal sections from prairie (*A*, *C*, and *E*) and montane (*B*, *D*, and *F*) vole brains. In the most anterior sections (*A* and *B*), binding to nucleus accumbens (Acc) and prelimbic cortex (PL) appears greater in the prairie vole (*A*). At the level of the crossing of the anterior commissure (*C* and *D*), binding to the BNST appears greater in the prairie vole (*C*), whereas the lateral septum (LSI) shows more labeling in the montane vole (*D*). At the most posterior level, binding to the midline thalamus (Th), ventral reuniens (VR), and lateral amygdala (AmL) is greater in the prairie vole (*E*); binding to the ventromedial nucleus of the hypothalamus (VMN) is greater in the montane vole (*F*); both species show high levels of binding to the central nucleus of the amygdala (AmC).

In contrast to OT, receptor maps for other neurotransmitters were relatively similar between species (Table 1). Binding for the benzodiazepine ligand [^{3}H]flunitrazepam was not statistically different in any brain area, including several regions with clear species differences in OT receptors. Binding of the μ-opioid receptor ligand [^{3}H]DAGO revealed species differences in only one region, the lateral septum, where binding was greater in prairie voles, in contrast to the pattern with ^{125}I-OTA binding. As with OT receptors, no sex differences were found with either benzodiazepine or μ-opioid receptor binding.

Comparative Studies. If OT receptor distribution were functionally related to social organization, a similar pattern should be evident in related species with analogous differences in behavior. Therefore, we next examined receptor distributions in two additional species of *Microtus*, the monogamous pine vole (*M. pinetorum*) (25) and the polygamous meadow vole (*M. pennsylvanicus*) (26). As with prairie and montane voles, ^{125}I-OTA binding in the lateral septum was

Table 1. Specific binding of ^{125}I-OTA, [^{3}H]flunitrazepam, and [^{3}H]DAGO in prairie and montane voles

^{125}I-OTA (oxytocin)				[^{3}H]flunitrazepam (benzodiazepine)				[^{3}H]DAGO (μ opioid)			
Area	Prairie	Montane	P	Area	Prairie	Montane	P	Area	Prairie	Montane	P
Acc	23.8 ± 5.7	3.6 ± 0.6	*	Acc	57.4 ± 1.5	53.8 ± 2.0	NS	Acc	117.3 ± 14.6	110.3 ± 7.6	NS
Prelimbic	22.7 ± 1.3	8.4 ± 2.1	**	Prelimbic	93.6 ± 3.6	87.3 ± 3.1	NS	Prelimbic	72.2 ± 6.5	60.8 ± 5.2	NS
LSI	9.9 ± 1.2	27.0 ± 2.8	***	LSI	67.4 ± 2.5	60.0 ± 3.0	NS	LSI	37.3 ± 2.1	22.5 ± 1.7	***
BNST	23.6 ± 3.9	7.4 ± 2.0	**	BNST	66.7 ± 3.9	71.7 ± 3.7	NS	BNST	ND	ND	
VMN	13.0 ± 3.0	22.0 ± 2.3	*	VMN	77.4 ± 1.2	73.1 ± 2.9	NS	VMN	38.3 ± 0.7	36.6 ± 0.8	NS
AmC	24.7 ± 2.9	21.3 ± 2.5	NS	AmC	107.9 ± 4.7	109.2 ± 3.5	NS	AmC	ND	ND	
AmL	20.2 ± 2.9	9.1 ± 1.6	**	AmL	91.8 ± 4.4	100.7 ± 9.4	NS	AmL	ND	ND	
VR	25.8 ± 6.1	3.8 ± 1.5	**	Ctx	106.8 ± 2.2	100.9 ± 8.0	NS	H-P	144.8 ± 22.3	90.3 ± 15.8	NS
Midline thal.	19.6 ± 2.8	5.5 ± 1.5	**	VP	120.0 ± 5.8	115.5 ± 3.9	NS	SN	53.0 ± 3.2	49.0 ± 1.0	NS
AmCO	14.7 ± 3.5	29.3 ± 3.5	*	Dentate	125.9 ± 6.6	135.5 ± 8.2	NS	AV	174.9 ± 15.6	143.3 ± 39.6	NS

Values are means (±SEM) of two to four determinations in each of six animals (three males and three females). Specific binding (total − nonspecific) is expressed as fmol per mg of protein. As gender differences were not observed by two-way ANOVA (species × sex), male and female values were combined and species differences were determined by independent t tests. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Acc, nucleus accumbens; LSI, lateral septal nucleus, intermediate; VMN, ventromedial hypothalamic nucleus; AmC, central amygdaloid nucleus; AmL, lateral amygdaloid nucleus; Ctx, cortex lamina 3 and 4; VP, ventral pallidum; VR, ventral reuniens thalamic nucleus; thal, thalamus; AmCO, posterior cortical amygdaloid nucleus; H-P, habenulointerpeduncular tract; SN, substantia nigra; AV, anteroventral thalamic nucleus; ND, not detectable.

intense in the polygamous species and relatively sparse in the monogamous species (meadow voles, 28.5 ± 2.6; pine voles, 3.8 ± 1.3 fmol per mg of protein; $t_{(8)} = 8.23$; $P < 0.001$) (Fig. 3). Unlike prairie and montane voles, these species showed no differences in ^{125}I-OTA binding in the nucleus accumbens or the BNST. Benzodiazepine receptor binding did not differ between these two species and [^{3}H]DAGO binding was not different in the lateral septum as observed for prairie and montane voles (data not shown).

Within-Species Experiments. Although these correlational results highlighted the differences in OT receptor distribution, these data failed to demonstrate that the receptor patterns were indeed related to the clear differences in social behavior. To investigate the relationship of receptor and behavioral differences.

We exploited a natural increase in affiliative behavior in the postpartum montane vole. After parturition, montane vole females changed from ignoring pups to showing active approach and care-taking behavior (assessed by time with pups in a Y-maze, females significantly increased their maternal behavior after parturition; $t_{(8)} = 2.99$; $P < 0.05$) (Fig. 4*A*). In contrast, naive (virgin) female prairie voles presented with pups for the first time showed high levels of maternal behavior that did not differ statistically from that of multiparous lactating females (Fig. 4*A*). The change in maternal behavior in the postpartum montane vole was associated with a significant increase in ^{125}I-OTA binding in the lateral amygdala (Fig. 4 *Bb* and *Bd*). In two separate experiments, binding in the lateral amygdala of lactating montane voles increased to 2–3.5 times the level seen in naive females (experiment 1, $t_{(4)} = 3.30$; $P < 0.05$; experiment 2, $t_{(4)} = 3.39$; $P < 0.05$) (Fig. 4*C*). The level of binding in the postpartum montane vole approximated the density observed in both naive and postpartum prairie voles (Fig. 4 *Ba*, *Bc*, and *C*). There were no differences in ^{125}I-OTA binding evident in any brain structure comparing naive and lactating female prairie voles.

DISCUSSION

These results demonstrate differences in the distribution of ^{125}I-OTA binding in prairie and montane voles that (*i*) are specific for OT receptors, (*ii*) are partially replicated in congeneric species, and (*iii*) appear to be associated with postpartum induction of affiliative behavior in montane voles. Several previous studies in rats and sheep have suggested that OT has effects in the brain that, consistent with its role in parturition and nursing, may subserve the initiation of social bonds. The induction of maternal (27–29), reproductive (30, 31), and grooming behaviors (32) and the reduction of pup separation distress (33) have all been demonstrated following central OT administration. The peptide may function physiologically to coordinate the early phases of these behaviors as OT antagonists or paraventric-

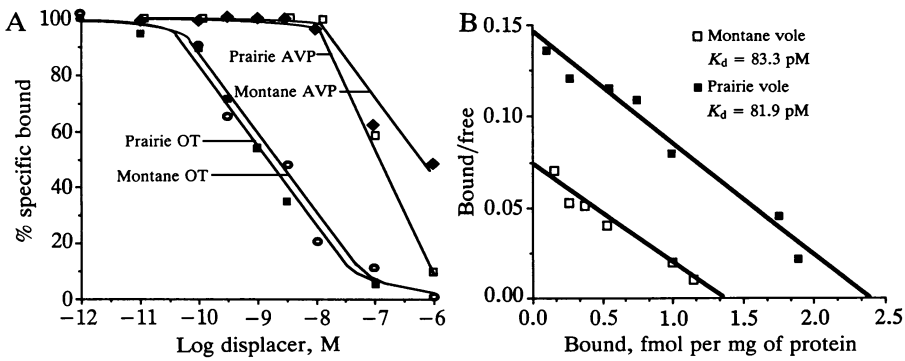

FIG. 2. Characterization of ^{125}I-OTA binding in homogenates from prairie and montane vole brains. (*A*) Displacement of ^{125}I-OTA binding (50 pM) with selective OT and AVP competitors was used to define the specificity of ^{125}I-OTA binding. Binding to OT receptors was defined by displacement with [Thr^4,Gly^7]OT, an OT agonist that binds very weakly to AVP receptors. Binding to AVP receptors was defined by displacement with the antagonist α-PTyr(Me)AVP, which binds selectively to the V_1 receptor. The >100-fold potency of the selective OT receptor for displacement of ^{125}I-OTA demonstrates that in both species the binding site resembles an OT receptor rather than a V_1 receptor. (*B*) Species differences in ^{125}I-OTA binding appear to be differences in B_{max} (number of receptors) rather than receptor affinity, as the K_d values are virtually equivalent in a saturation study with ^{125}I-OTA and unlabeled OT used as a displacer.

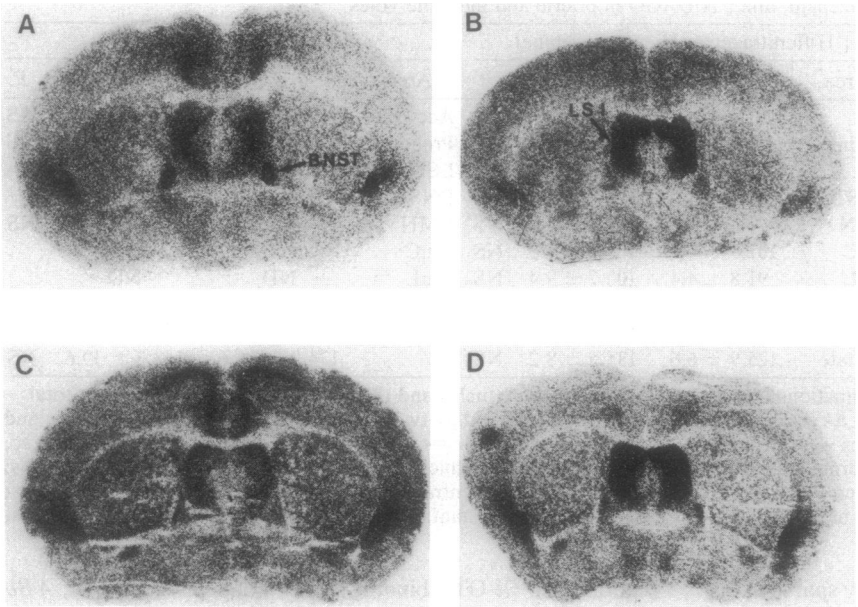

FIG. 3. Representative autoradiograms of ^{125}I-OTA binding to 20-μm coronal sections from prairie vole (*A*), montane vole (*B*), pine vole (*C*), and meadow vole (*D*). These additional species were selected to replicate some of the behavioral differences shown by prairie and montane voles. Pine voles appear monogamous on several measures but are less paternal than prairie voles. Meadow voles resemble montane voles in social organization, inhabiting isolated burrows, and appearing polygamous on most laboratory measures. Each section is from a naive male 45–90 days old. OT receptors appear dense in the lateral septum of both polygamous species (*B* and *D*). Binding to the cingulate cortex is high in the pine vole, similar to the pattern observed in the prelimbic region more rostrally in the prairie vole.

ular nucleus lesions decrease the initiation but not the maintenance of maternal (34–36) and reproductive behaviors (37). The recent finding that OT receptors increase in specific brain areas with parturition and estrus provides a cellular mechanism by which OT might induce the onset of maternal behavior and/or sexual receptivity (18).

The specific functional significance of the neuroanatomical areas highlighted by ^{125}I-OTA binding in the present study must be inferred from previous studies in other species of rodents. The high level of ^{125}I-OTA binding in the BNST and amygdala of the highly parental prairie vole is consistent with previous results demonstrating a relationship between OT receptors in the BNST and the onset of maternal behavior in the rat (19). In the female rat, which normally avoids conspecific pups, the onset of maternal behavior occurs at parturition in association with an 87% increase in ^{125}I-OTA binding in the BNST but not in several other brain regions (19). Relative to several other OT receptor-rich regions, the BNST includes a high density of estrogen-concentrating cells (38) and the physiologic changes in gonadal steroids at parturition appear sufficient to increase OT receptors in this region (39). By contrast, in the female prairie vole, which is highly parental outside of the postpartum period (Fig. 2), high concentrations of OT receptors are apparent in the BNST, even in the absence of gonadal steroids (40).

Behavioral studies further implicate OT in the mediation of the prairie vole's social behavior. The female prairie vole, which does not manifest estrous cycles, remains sexually inactive until stimulated by male chemosignals (41). Within 24–48 hr of male exposure, the female becomes sexually receptive and after prolonged bouts of mating, forms a lasting social bond as manifested by persistent physical proximity with her mate and frequent aggression toward other conspecifics (9). OT is released centrally during mating (42, 43) and central (but not peripheral) injections of OT induce a dose-dependent increase in partner preference (44) as well as increased grooming and side-by-side proximity in prairie voles (45). A working hypothesis is that OT released during mating activates those limbic sites rich in OT receptors to confer some lasting and selective reinforcement value on the mate. Several of the regions rich in OT receptors (nucleus accumbens, lateral amygdala, prelimbic cortex) have been

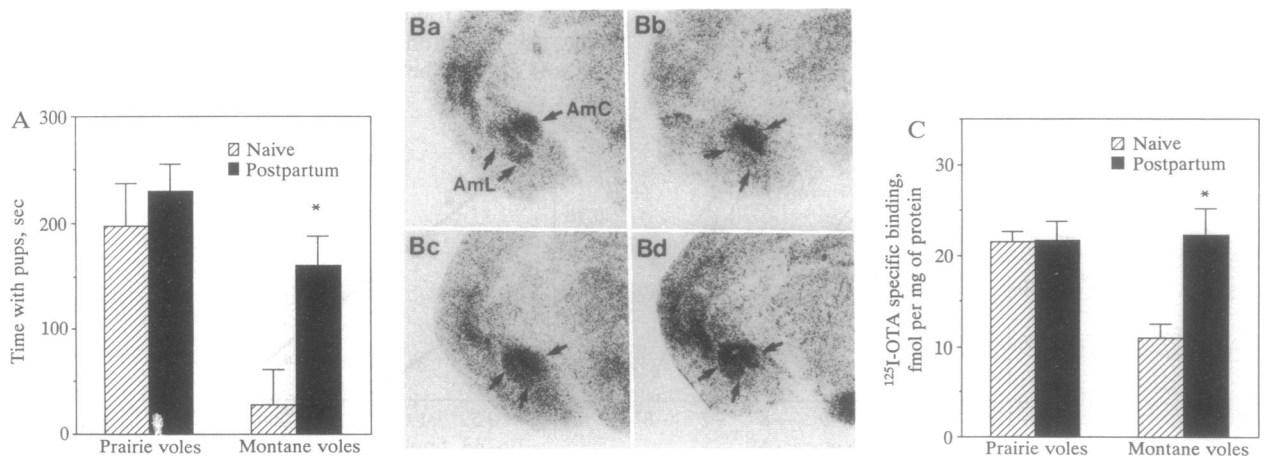

FIG. 4. Changes in behavior and ^{125}I-OTA binding postpartum in prairie and montane voles. (*A*) The prairie vole is highly parental in both the naive (virgin) and lactating state ($t_{(10)} = 0.77$; not significant), whereas the montane vole approaches pups significantly more postpartum ($t_{(8)} = 2.99$; $P < 0.05$). (*B*) These behavioral changes are associated with differences in ^{125}I-OTA binding in the montane female. Binding in the lateral amygdala (AmL) does not change in the prairie vole from the naive (*Ba*) to the postpartum (*Bc*) state, whereas, in the montane vole, binding in this same region increases within 24 hr postpartum [*Bb* (naive) vs. *Bd* (postpartum) montane female]. Note the absence of a concurrent increase in binding to the central nucleus of the amygdala. (*C*) Quantitative analysis of autoradiograms shows that ^{125}I-OTA binding in the lateral amygdala increases ≈100% in the postpartum montane vole ($t_{(4)} = 3.30$; $P < 0.05$). *, $P < 0.05$ within species.

implicated in the development of associations (46–49), but the role of OT in any specific region in the prairie vole brain remains to be determined.

The intense binding seen in the lateral septum both less-affiliative species is a finding of particular interest. Although septal lesions in rats have been shown to increase aggression, disrupt maternal behavior, and suppress male sexual behavior, the septum's role in the mediation of affiliation is complex and not easily generalized across species (50–52). Indeed, septal lesions increased social contact time in one subspecies of deer mouse (*Peromyscus maniculatus gracillis*) while decreasing contacts in a less-social subspecies (*Peromyscus maniculatus bairdi*) (53). OT effects in montane voles (or meadow voles) have not been studied extensively, but preliminary data from our own group demonstrate that in a social interaction test, montane voles show remarkably little behavioral response to central administration of 50 ng of OT, a dose that increases defensive aggression as well as conferring several other effects described above for prairie voles [J. T. Winslow and T.R.I., unpublished data (available upon request)].

It should be noted that the failure to entirely replicate the prairie vole/montane vole differences in OT receptor distribution in the pine/meadow vole comparison could be due to incomplete parallelism in the behavioral differences between the two pairs of microtine species. For instance, pine voles are monogamous by most criteria (7) but show significantly less parental care than prairie voles (8). Just as maternal and reproductive behaviors may be mediated by separate OT receptor fields in the rat brain, results with pine and meadow voles suggest that OT receptors in the septum may be associated with social organization (i.e., monogamy vs. polygamy), whereas OT receptors in the BNST/amygdala may be important for parental behavior, which is highest in prairie voles. We have previously reported species differences in OT receptor distribution in the septum in a comparison of monogamous and polygamous mice (54).

While we have observed differences in the neuroanatomic distribution of OT receptors, the physiologic role of OT in these brain regions remains unknown. Physiologic data on the function of central OT receptors will be vital for understanding how this OT system modulates the processing of sensory information. Similarly, we have no information on the structural or molecular characterization of this receptor with which to judge whether this is a single protein across microtine species or whether several different OT receptors exist.

The results of correlational studies must be interpreted with caution. Nevertheless, in light of the emerging body of work on this neuropeptide system, the present comparative findings support the hypothesis that the distribution of OT receptors may be an important substrate for those ecological pressures selecting for differences in the affective/motivational processing of social stimuli—differences that at the level of the population emerge as contrasting systems of social organization.

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