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Short communication

Ontogeny of oxytocin and vasopressin receptor binding in the lateral septum in prairie and montane voles

Zuoxin Wang *, Larry J. Young

Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, 1639 Pierce Drive, Atlanta, GA 30322 USA

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Abstract

Adult prairie (*Microtus ochrogaster*) and montane voles (*M. montanus*) differ in the distribution of oxytocin (OT) and vasopressin (AVP) receptor binding in the brain. The present study examined the ontogenetic pattern of these receptor bindings in the lateral septum in both species to determine whether adult differences in the receptor binding are derived from a common pattern in development. In both species, OT and AVP receptor binding in the lateral septum were detected neonatally, increased during development, and reached the adult level at weaning (third week). The progression of OT and AVP receptor differed, as OT receptor binding increased continually until weaning while AVP receptor binding did not change in the first week, increased rapidly in the second week, and was sustained thereafter. For both receptors, the binding increased more rapidly in montane than in prairie voles, resulting in species differences in receptor binding at weaning and in adulthood. Together, these data indicate that OT and AVP could affect the brain during development in a peptide- and species-specific manner in voles. © 1997 Elsevier Science B.V.

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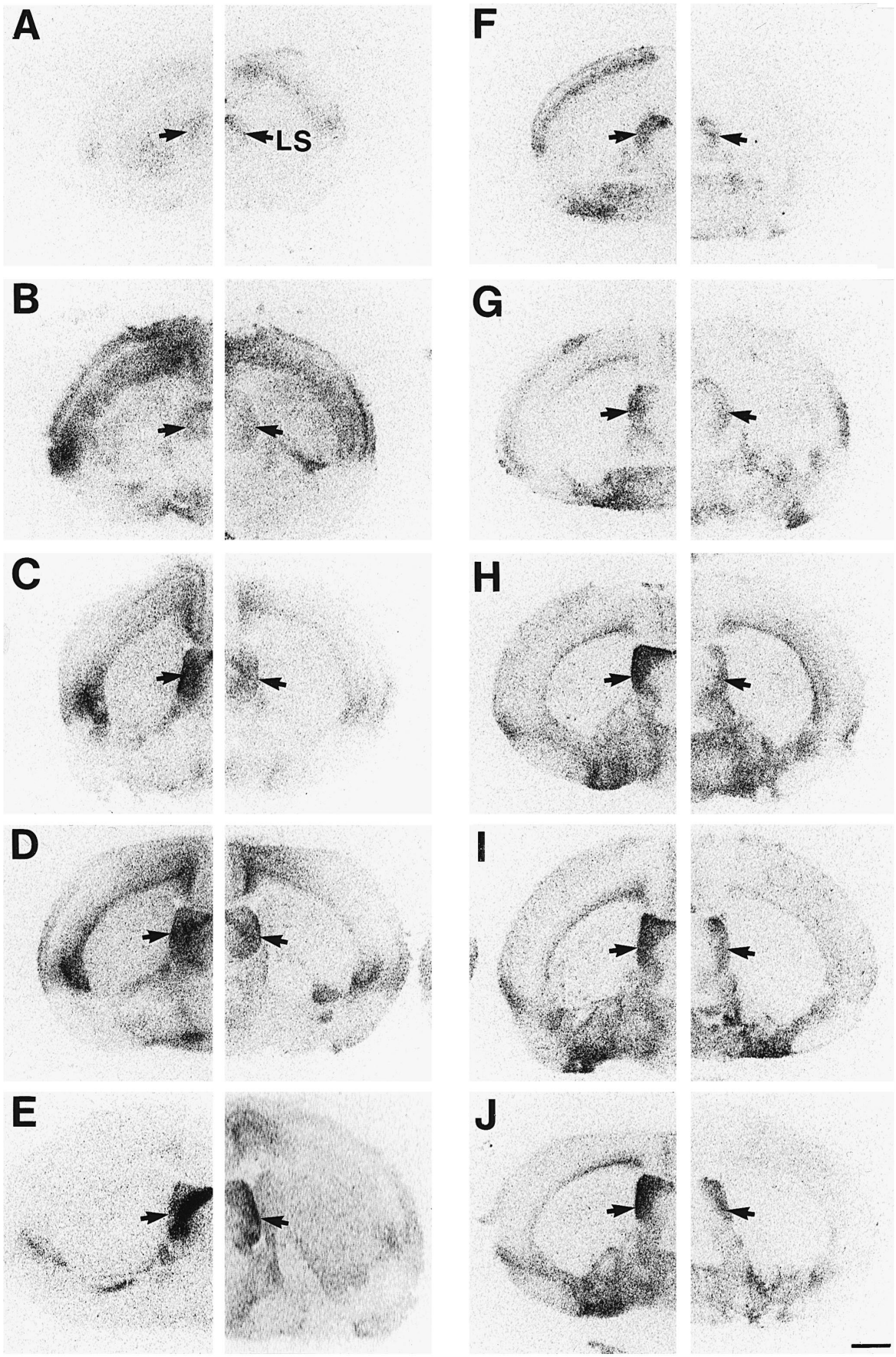
Membrane-bound oxytocin (OT) and vasopressin (AVP) V_{1a} receptors are distributed in the central nervous system, where they regulate OT or AVP functions in physiology and behavior in rodents [4,5,9,20,31]. In monogamous prairie voles (*Microtus ochrogaster*), OT is implicated in the formation of stable pair bonds in females [12,30] whereas AVP affects pair bonds and paternal behavior in males [27,31]. Recently, OT and AVP receptor binding in prairie voles has been found to be remarkably different from promiscuous montane voles (*M. montanus*) that are taxonomically closely related but differ in social organization and behavior. The two species differ in the distribution pattern and regional quantity of OT and AVP receptors but exhibit similar patterns in the benzodiazepine or opioid receptors [13,14]. The species differences in OT and AVP receptor binding are due to differences in region-specific gene expression [33,34]. In a more recent study, AVP receptor binding showed an early appearance, transient expression and redistribution during postnatal development in male voles, suggesting that AVP may function differently in infant and adult brains [28]. The ontogenetic pattern of OT receptor distribution in voles is still unknown.

In the present study, we examined the postnatal development of OT and AVP receptors in the female prairie and montane vole using receptor autoradiography. We tested the hypothesis that adult species differences in OT and AVP receptor binding derive from a common pattern in development. This study was focused on the lateral septum because this area contains a species-specific level of OT and AVP receptor binding in voles [13,14], and septal OT and AVP has been implicated in social behavior and reproduction in rodents [6,8,16,18,19,27].

Subjects were the female offspring of prairie and montane voles from our breeding colony. Animals were decapitated on the day of birth, or at 1 week, 2 weeks, 3 weeks, or 3 months of age ($n = 5$ per age per species). Brains were removed, frozen on dry ice, cut into 20 μm sections on a cryostat, and sections through the lateral septum were thaw-mounted on Superfrost/plus slides. One set of sections at 100 μm intervals was processed for OT receptor autoradiography whereas another set was processed for AVP receptor autoradiography. The detailed procedures for OT and AVP receptor binding, characterization of the

* Corresponding author. Fax: +1 (404) 727-3233; e-mail: zwang@emory.edu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^{125}I-OTA and ^{125}I-linear-AVP ligands (Dupont, Boston, MA), and data quantification have been described previously [13,28]. In the current experiment, the tracer was 50 pM of ^{125}I-OTA or ^{125}I-linear-AVP, respectively. To define non-specific binding, adjacent sections were incubated in the incubation buffer containing either ^{125}I-OTA pretreated with 1 μm selective OT antagonist, [Thr^{4}Gly^7]OT, or ^{125}I-linear-AVP pretreated with 1 μm the V_{1a} ligand, d(CH$_2$)$_5$[Tyr(Me)]AVP. In each case, the specific binding was displaced. In a previous study, pre-treatment of ^{125}I-linear-AVP with AVP or the V_{1a} antagonist displaced the specific binding whereas pre-treatment with AVP V_2 antagonist, OT, or OT antagonist did not influence binding, indicating that ^{125}I-linear-AVP binds specifically to AVP V_{1a} receptors in voles [28]. Slides from both species were processed simultaneously to reduce variability. OT or AVP receptor binding in the lateral septum was measured bilaterally in four sections for each subject to provide individual mean. Data for each receptor binding were analyzed by a two-way ANOVA (Species × Age). Significant age and species by age effects were further evaluated by a Student-Newman-Keul's post hoc test.

The ^{125}I-OTA binding was displaced by the OT antagonist, suggesting that the ligand bound selectively to OT receptors. This selectivity was evident in adults and infants of both species. A low level of ^{125}I-OTA binding in the lateral septum was found neonatally. The binding increased continuously during postnatal development until it reached the adult level at weaning (3 weeks; $F = 44.1$, $df = 4/38$, $P < 0.001$; Figs. 1 and 2a). This increase in ^{125}I-OTA binding was in a species-specific manner ($F = 5.0$, $df = 4/38$, $P < 0.01$). In the two species, the binding increased equally in early development, and then increased more in montane than in prairie voles. Consequently, montane voles had a higher level of OT receptor binding at weaning and in adulthood relative to prairie voles.

Specific ^{125}I-linear-AVP binding was displaced by the V_{1a} antagonist in both infant and adult brains [28]. A low level of ^{125}I-linear-AVP binding in the lateral septum was detected neonatally in both species. Such binding remained unchanged in the first week, increased rapidly in the second week, and sustained thereafter ($F = 36.6$, $df = 4/38$, $P < 0.001$; Figs. 1 and 2b). This developmental change of AVP receptor binding was species-specific ($F = 8.5$, $df = 4/38$, $P < 0.01$). The two species did not differ in the binding at birth and in the first week of age. In the second week, the binding increased more in montane than in prairie voles, resulting a species difference.

In previous studies in rats, OT and AVP receptor binding in the lateral septum appeared neonatally, increased during development and reached the adult level at weaning [21,25,26]. A similar ontogenetic pattern was also found in voles in the present study. However, our data indicate that OT and AVP receptor binding developed in a species-specific manner, and adult differences in the receptor binding were derived from a common pattern in development. Neither OT nor AVP receptor binding in the lateral septum differed neonatally between the two species. Thereafter, the developmental increase was more accelerated in montane than prairie voles, resulting species differences in OT and AVP receptor binding at weaning. This difference may result from species differences in genesis and death of neuronal cells containing OT or AVP receptors.

Our data also indicate a different developmental profile between AVP and OT receptor binding in the lateral septum. AVP receptor binding was unchanged in the first week, substantially increased in the second week, and

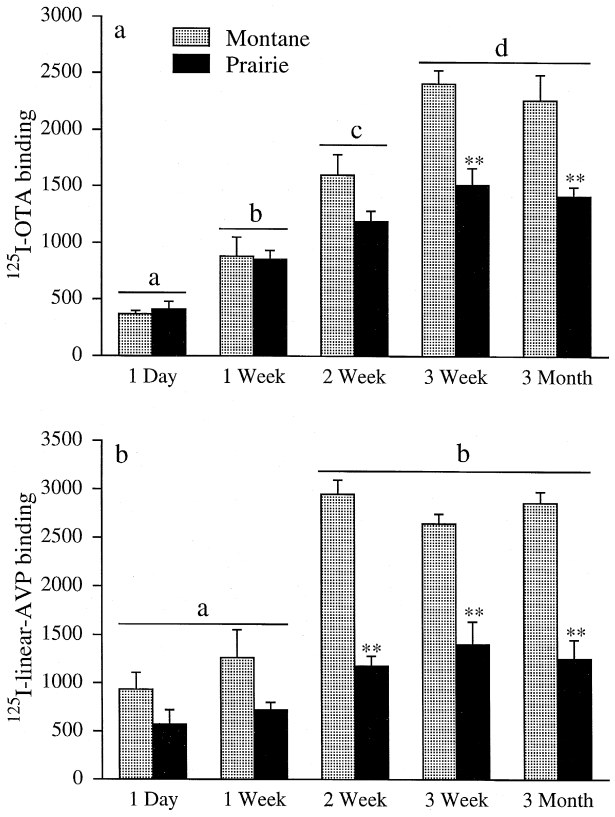

Fig. 2. The ^{125}I-OTA (a) and ^{125}I-linear-AVP (b) binding in the lateral septum in montane (dotted bars) and prairie voles (solid bars) during development. Data were presented as dpm/mg tissue equivalent and analyzed by a two-way (Age × Species) ANOVA followed by a Student-Newman-Keul's post hoc test. The alphabetic letters represent significant age differences whereas the asterisks indicate species differences within each age group. Bars: means ± SEM.

Fig. 1. Photomicrographs displaying ^{125}I-OTA (A–E) and ^{125}I-linear-AVP binding (F–J) in the lateral septum (LS) in prairie voles (right of each composed section) and montane voles (left of each composed section) on the day of birth (A,F) and at 1 week (B,G), 2 weeks (C,H), 3 weeks (D,I), and 3 months of age (E,J). Scale bar: 1 mm.

sustained thereafter. However, OT receptor binding showed a continuous increase and reached the adult level at weaning. Our finding is consistent with the finding in rats, in which AVP receptor binding reached the adult level at 2 weeks whereas OT receptor binding developed with a lag time of 1 week [23,25,26]. It may be that some physiological factors at a critical period in early life triggered development of AVP receptors, whereas the regulation of OT receptors was more dependent upon other factors that changed continuously during development and reproduction. This notion is supported by the fact that the adult pattern of AVP receptor binding generally is not subjected to further changes (but see [17]) whereas OT receptor binding changes during puberty and reproduction and is regulated by gonadal steroids in several species of rodents [11,15,21,24–26,32]. The pattern of the receptor ontogeny is also parallel to the developmental pattern of peptide producing cells, in which the presence of *OT* gene or OT innervation in the rat brain was late with respect to AVP [1,3]. In addition, the adult pattern of AVP immunoreactive fiber plexuses in the lateral septum was detected only around the postnatal day 14 and onward [7], indicating parallel developmental profiles between AVP fibers and receptors in rats. OT fibers were not found in the lateral septum in rats and voles [2,29].

In short, the developmental pattern of OT and AVP receptor binding in the present study indicate that OT and AVP may affect the brain and behavior during development in a species- and peptide-specific manner in voles. Indeed, monogamous and promiscuous infant voles differ in the postnatal brain development [10] as well as in the behavioral and physiological responses to social isolation [22]. The possible involvement of OT and AVP in these functions needs to be further studied.

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