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Species Differences in Vasopressin Receptor Binding Are Evident Early in Development: Comparative Anatomic Studies in Prairie and Montane Voles

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ABSTRACT

Monogamous prairie voles (*Microtus ochrogaster*) and promiscuous montane voles (*Microtus montanus*) exhibit remarkable differences in the distribution of vasopressin (AVP) receptors in the adult brain. This difference in receptor distribution is associated with species differences in the behaviors, including pair bond formation and paternal care, found selectively in the monogamous vole. To investigate a potential mechanism for this species difference in AVP receptors, the present study examined the ontogeny of receptor binding in the two species to determine whether the adult maps arose from a shared pattern in development. By using ^{125}I-linear-AVP, which is a selective high-affinity ligand for the V_{1a} receptor, we found early appearance and transient expression of AVP receptor binding during postnatal development in both species. However, the ontogenetic patterns of regional AVP receptor binding were species specific. In the diagonal band, the bed nucleus of the stria terminalis, and the central nucleus of the amygdala, prairie voles had higher AVP receptor binding at birth than montane voles, and this difference persisted with little variation into adulthood. In these areas, therefore, species differences in AVP receptor binding appeared to be determined primarily by genetic or prenatal factors. In the lateral septum, both species had low levels of AVP receptor binding at birth. Thereafter, the binding increased rapidly in montane voles, but it remained unchanged in prairie voles. In the cingulate cortex, AVP receptor binding in prairie voles showed a peak in early development with a subsequent decline and reached the adult level at weaning, whereas the binding in montane voles remained unchanged into adulthood. A similar but opposite pattern was found in the frontoparietal cortex, in which AVP receptor binding showed an early peak in montane voles but did not change significantly in prairie voles. These results demonstrate that 1) species differences in regional AVP receptor binding are evident in the early postnatal period and, in several areas, may be determined by genetic or prenatal factors, and 2) AVP may target brain areas differently in infant and adult prairie and montane voles and, thus, could exert differential effects on the organization of the central nervous system in the two species of voles. J. Comp. Neurol. 378:535–546, 1997.

Indexing terms: lateral septum; amygdala; monogamy; V_{1a}

In the central nervous system, vasopressin (AVP) is primarily synthesized in several hypothalamic areas, including the paraventricular, supraoptic, and suprachiasmatic nuclei as well as the bed nucleus of the stria terminalis (BST) and the medial amygdala (AMYG; Buijs et al., 1978; van Leeuwen and Caffé, 1983; Sherman et al., 1986; De Vries and Al-Shamma, 1990). AVP synthesized in the hypothalamic nuclei is released via the posterior pituitary into the bloodstream, and its peripheral role in the maintenance of fluid homeostasis and blood pressure has been well documented (Cowley and Liard, 1987; Valtin, 1987). In addition, AVP-synthesizing cells from both hypothalamic and extrahypothalamic areas project

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into various central targets (Buijs et al., 1978; De Vries et al., 1981, 1985; Caffé et al., 1987), where AVP acts as a neurotransmitter or neuromodulator to regulate physiological and behavioral functions, such as body temperature (Meisenberg and Simmons, 1983; Wilkinson and Kasling, 1987), blood pressure (Pittman et al., 1982), brain development (Boer et al., 1980, 1982), memory (de Wied, 1977; Dantzer et al., 1988; Bluthe et al., 1990), and social behaviors (Ferris et al., 1984; Koolhaas et al., 1990; Albers et al., 1992; Winslow et al., 1993; Wang et al., 1994a).

The actions of AVP are mediated by three subtypes of membrane-bound receptors, namely, V_{1a}, V_{1b}, and V_2 receptors (for review, see Barberis and Tribollet, 1996), with V_{1a} predominating in the central nervous system (Jard, 1983; van Leeuwen et al., 1987). Early studies that used tritiated (^{3}H-AVP) or iodinated AVP ligands (^{125}I-sarc-AVP) with in vitro autoradiography have localized brain AVP receptors in several species of rodents (Biegon et al., 1984; van Leeuwen et al., 1987; Freund-Mercier et al., 1988a,b; Phillips et al., 1988; Tribollet et al., 1988; Gerstberger and Fahrenholz, 1989; Dubois-Dauphin et al., 1991; Insel et al., 1994). Recently, an iodinated linear AVP ligand (^{125}I-linear-AVP) has been developed, and its high affinity (approximately 60 pM) and selectivity for V_{1a} receptors make this ligand an excellent tool for studies on central AVP receptor localization and characterization (Schmidt et al., 1991; Johnson et al., 1993; Barberis et al., 1995).

In a previous study, we found that brain AVP receptor binding in microtine rodents showed remarkable species differences, which appeared to be associated with the pattern of social organization. Monogamous prairie voles (*Microtus ochrogaster*) and pine voles (*M. pinetorum*) differed from promiscuous montane voles (*M. montanus*) and meadow voles (*M. pennsylvanicus*) in most regions, showing specific binding of either ^{3}H-AVP or ^{125}I-sarc-AVP (Insel et al., 1994). Monogamous and promiscuous voles differ in several patterns of social behaviors, such as partner preference (Shapiro and Dewsbury, 1990; Carter and Getz, 1993), mating-induced aggression (Carter et al., 1986; Insel et al., 1995), and paternal behavior (Hartung and Dewsbury, 1979; Oliveras and Novak, 1986). Pharmacologic studies with AVP and a selective V_{1a} antagonist have implicated this receptor in the modulation of these behaviors in monogamous voles (Winslow et al., 1993; Wang et al., 1994a), suggesting that species differences in the distribution of V_{1a} receptors may be important for the control of social behaviors associated with differences in vole reproductive strategy.

The present study was undertaken to investigate the development of these species differences in V_{1a} receptors. In rat brain, AVP receptor binding shows early appearance and transient expression during postnatal development (Petracca et al., 1986; Snijdewint et al., 1989; Tribollet et al., 1991). We tested two alternative hypotheses regarding the postnatal development of these receptors in voles. First, adult species differences in receptor distribution could arise from a common pattern in development, with the two species diverging at a critical point in development, such as weaning or puberty. Alternatively, the two species could differ throughout development, suggesting that genetic or prenatal factors regulate their expression differentially. We compared AVP receptor binding at different stages of development between monogamous prairie voles and promiscuous montane voles by using ^{125}I-linear-AVP. This newly developed, high-affinity, selective V_{1a} ligand has been reported to detect AVP receptors in some rat brain regions that could not be detected by other AVP ligands (Schmidt et al., 1991; Johnson et al., 1993; Barberis et al., 1995). Therefore, in addition to the developmental study, we repeated our previous study comparing adults of both species, this time using ^{125}I-linear-AVP ligand.

Abbreviations

ac	anterior commissure
AVP	vasopressin
BST	bed nucleus of the stria terminalis
BSTad	bed nucleus of the stria terminalis (dorsal)
CA3	field CA3, stratum
Ce	central nucleus of amygdala
Ctx	cortex (frontoparietal)
DB	diagonal band
DLG	dorsolateral geniculate nucleus
LH	laterodorsal hypothalamic area
LP	lateroposterior thalamus
LD	laterodorsal thalamus
LS	lateral septum
LSD	lateral septum, dorsal
MD	mediodorsal thalamus
Me	medial nucleus of amygdala
MG	medial geniculate nucleus
NAc	nuclear accumbens
PCg	postcingulate cortex
Po	posterior thalamus
PT	paratenial thalamus
Rt	reticular thalamus
TT	taenia tecta
VM	ventromedial hypothalamus
VP	ventroposterior thalamus

MATERIALS AND METHODS

Subjects

Subjects in the present study were the offspring of the laboratory breeding colony, which was derived from field-captured prairie voles (*M. ochrogaster*) and montane voles (*M. montanus*). The breeding pairs for each species were housed in plastic cages (44 × 24 × 20 cm) that contained cedar chip bedding. Water and food were provided ad libitum. The cages were maintained on a 14:10 hours light:dark photoperiod, with lights on at 0700 hours. The temperature in the colony room was about 20°C. The colony was checked daily, and the male offspring from each species were decapitated on the day of birth and at 1 week, 2 weeks, or 3 weeks of age. In addition, some offspring were weaned at 3 weeks of age, housed in same-sex sibling pairs in plastic cages, and maintained under the same conditions as their parents. These subjects were decapitated at 3 months of age. Only males were studied in this series, because central AVP receptor binding in adult voles showed no sex differences (Insel et al., 1994), and central AVP has effects on social behaviors in male voles (Winslow et al., 1993; Wang et al., 1994a; central AVP effects on social behaviors have not been studied in female voles). After decapitation, brains were removed quickly, frozen on dry ice, and stored at −80°C. To avoid the "litter effect," no more than two subjects within each age group were from the same litter. Brains were cut into 20 μm sections on a cryostat and thaw mounted on Superfrost/Plus slides (Fisher). The use of animals and methods in this study met the National Institutes of Health (NIH) guidelines and

were approved by the Animal Care and Use Committee at Emory University.

AVP receptor autoradiography

Slide-mounted sections at 100 µm intervals were thawed for 30 minutes at room temperature and were preincubated in 50 mM Tris-HCl buffer, pH 7.4, twice for 5 minutes each. Sections were then placed in incubation buffer consisting of 50 mM Tris-HCl, pH 7.4, with 10 mM $MgCl_2$, 0.1% bovine serum albumin (BSA), 0.05% bacitracin, and 50 pM tracer for 60 minutes at room temperature. The tracer was ^{125}I-linear-AVP ligand [Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2; New England Nuclear-Dupont, Boston, MA], which was previously characterized in rats (Schmidt et al., 1991; Johnson et al., 1993). Following incubation, sections were lightly fixed in 50 mM Tris-HCl with 10 mM $MgCl_2$ and 0.1% paraformaldehyde for 2 minutes at room temperature (to protect tissue integrity), washed in 50 mM Tris-HCl with 10 mM $MgCl_2$ four times for 5 minutes each at 4°C, and then washed in the same solution with stirring for 30 minutes at room temperature. Sections were dipped in water and then immediately dried under a stream of cool air. In adjacent sections from the subjects of each age group, 1 µM of the selective V_{1a} ligand d$(CH_2)_5$[Tyr(Me)]AVP was added to the incubation buffer to define nonspecific binding. In addition, to characterize the specificity of ^{125}I-linear-AVP in voles, in adjacent brain sections (20 µm) from 1-week-old, 2-week-old, and adult voles of both species, 1 µM AVP, selective V_{1a} ligand d$(CH_2)_5$[Tyr(Me)]AVP, V_2 ligand [d$(CH_2)_5$,D-Phe^2,Ile^4,Ala^9-NH_2]-AVP, oxytocin (OT), or selective OT ligand [Thr^{4}Gly^7]OT were used as competitors.

After drying, the slides were exposed to a BioMax MR film (Kodak) along with ^{125}I plastic standards ([I-125] microscales; Amersham, Chicago, IL) for 2 days. The resulting autoradiograms were analyzed with a computerized image program (IMAGE 1.57 developed by Dr. W. Rasbard at NIH), permitting the conversion of optical density to dpm/tissue equivalents from the standard curve derived from coexposed standards. Nonspecific binding was subtracted from total binding to yield values for specific binding.

Data analysis

All slides from both species were processed simultaneously for AVP receptor binding. Diagrammatic representative brain sections from Paxinos and Watson (1986) were used to define anatomic regions. Two to three sections were measured for each brain region in each subject to provide individual means. Developmental changes of AVP receptor binding were analyzed by using a two-way analysis of variance (ANOVA) with species and age as between-subject variables. Significant age effect and species by age interaction were further evaluated by using a Newman Keuls post hoc test (SNK). In addition, species differences in regional AVP receptor binding in adult voles were analyzed by using a Student's t test.

RESULTS

The forebrains of adult prairie and montane voles showed specific binding for ^{125}I-linear-AVP. The pattern of this binding essentially replicated the pattern described using ^{125}I-sarc-AVP (Insel et al., 1994) and extended the previous observation by revealing more brain areas that contain AVP receptors (Table 1; Figs. 1, 2). For example, in addition to previously reported brain areas that contain AVP receptors (Insel et al., 1994), ^{125}I-linear-AVP binding was detected in taenia tecta, endopiriform nucleus, medial nucleus of the AMYG, periaqueductal gray, and supramammillary nucleus. Adult prairie and montane voles did not show different AVP receptor binding in these areas. However, in the paratenial and reticular thalamus, the ventromedial hypothalamus, and the medial mammillary nucleus, montane voles had higher ^{125}I-linear-AVP binding than prairie voles. In contrast, prairie voles had higher ^{125}I-linear-AVP binding in the laterodorsal hypothalamus than montane voles.

TABLE 1. ^{125}I-Linear-Vasopressin Binding in Adult Prairie and Montane Voles (Mean ± S.E.M. dpm/Tissue Equivalent)

Brain region	Abbreviation	Prairie vole	Montane vole
Taenia tecta	TT	6,034 ± 510	5,330 ± 348
Nucleus accumbens	NAc	362 ± 63	356 ± 26
Diagonal band	DB	7,341 ± 872	4,846 ± 235*
Endopiriform nucleus (dorsal)	EPd	3,968 ± 590	5,425 ± 477
Lateral septum	LS	2,961 ± 329	7,913 ± 579**
Bed nu. stria terminalis (dorsal)	BSTad	2,504 ± 152	1,388 ± 131**
Bed nu. stria terminalis (ventral)	BSTav	6,102 ± 621	3,329 ± 229**
Cingulate cortex (posterior)	PCg	4,443 ± 1,393	1,880 ± 312
Frontoparietal cortex	Ctx	353 ± 41	791 ± 162*
Habenula	Hb	1,560 ± 675	1,219 ± 153
Thalamus (paratenial)	PT	9,992 ± 1,590	26,194 ± 2,715**
Thalamus (paracentral)	PC	2,502 ± 708	4,904 ± 1,497
Thalamus (mediodorsal)	MD	9,065 ± 863	5,373 ± 688*
Thalamus (laterodorsal)	LD	22,344 ± 3,852	3,062 ± 1,278**
Thalamus (posterior)	Po	5,808 ± 888	8,024 ± 1,457
Thalamus (reticular)	Rt	1,028 ± 179	6,475 ± 417**
Thalamus (centromedial)	CM	4,007 ± 345	2,181 ± 198**
Thalamus (lateroposterior)	LP	12,896 ± 993	11,665 ± 1,428
Thalamus (ventroposterior)	VP	4,900 ± 694	18,961 ± 2,423**
Central nucleus of amygdala	Ce	4,890 ± 399	1,555 ± 214**
Medial nucleus of amygdala	Me	5,119 ± 719	5,972 ± 375
Hypothalamus (ventromedial)	VM	5,468 ± 474	9,157 ± 381**
Hypothalamus (laterodorsal)	LH	4,144 ± 194	3,112 ± 127**
Dorsolateral geniculate nucleus	DLG	17,520 ± 4,124	13,400 ± 2,134
Medial geniculate nucleus	MG	14,106 ± 2,822	10,293 ± 1,120
Dentate gyrus (lateral)	DG	10,135 ± 1,001	4,365 ± 320**
Periaqueductal gray	PAG	4,855 ± 162	4,066 ± 437
Supramammillary nucleus (medial)	SUMm	2,687 ± 424	4,767 ± 1,056
Medial mammillary nucleus	MM	542 ± 143	7,523 ± 1,171**
Field CA3, stratum	CA3	1,588 ± 245	8,900 ± 726**

*$P < 0.05$.
**$P < 0.01$ (means were compared by using a t test).

Binding of ^{125}I-linear-AVP in both species was completely displaced by AVP or by the V_{1a} antagonist but was not displaced by a V_2 ligand, by OT, or by the OT antagonist (Fig. 3). Thus, in both species, ^{125}I-linear-AVP appeared to bind selectively to a V_{1a} receptor. This selectivity was evident in both adults and infants of both species.

To study ^{125}I-linear-AVP binding during development, we focused on the diagonal band (DB), the lateral septum (LS), the BST, the AMYG, the cortex, and the hypothalamic areas that could be reliably defined in both adult and infant brains. Although extensive ^{125}I-linear-AVP binding was found in the thalamus in adults of both species, difficulty in determining the boundaries of specific thalamic regions in the infant brain, especially at 1 day or 1 week of age, prohibited us from reliably measuring V_{1a} receptors in those areas.

^{125}I-linear-AVP binding showed early appearance, transient expression, and redistribution in both species in a regionally specific manner. In addition, developmental patterns of regional binding differed between the two species. Several general patterns were observed. In the DB (Fig. 4a), the ventral nucleus of the BST (Figs. 4c, 5), and the central nucleus of the AMYG (Figs. 4e, 6), prairie voles had higher ^{125}I-linear-AVP binding at birth than montane

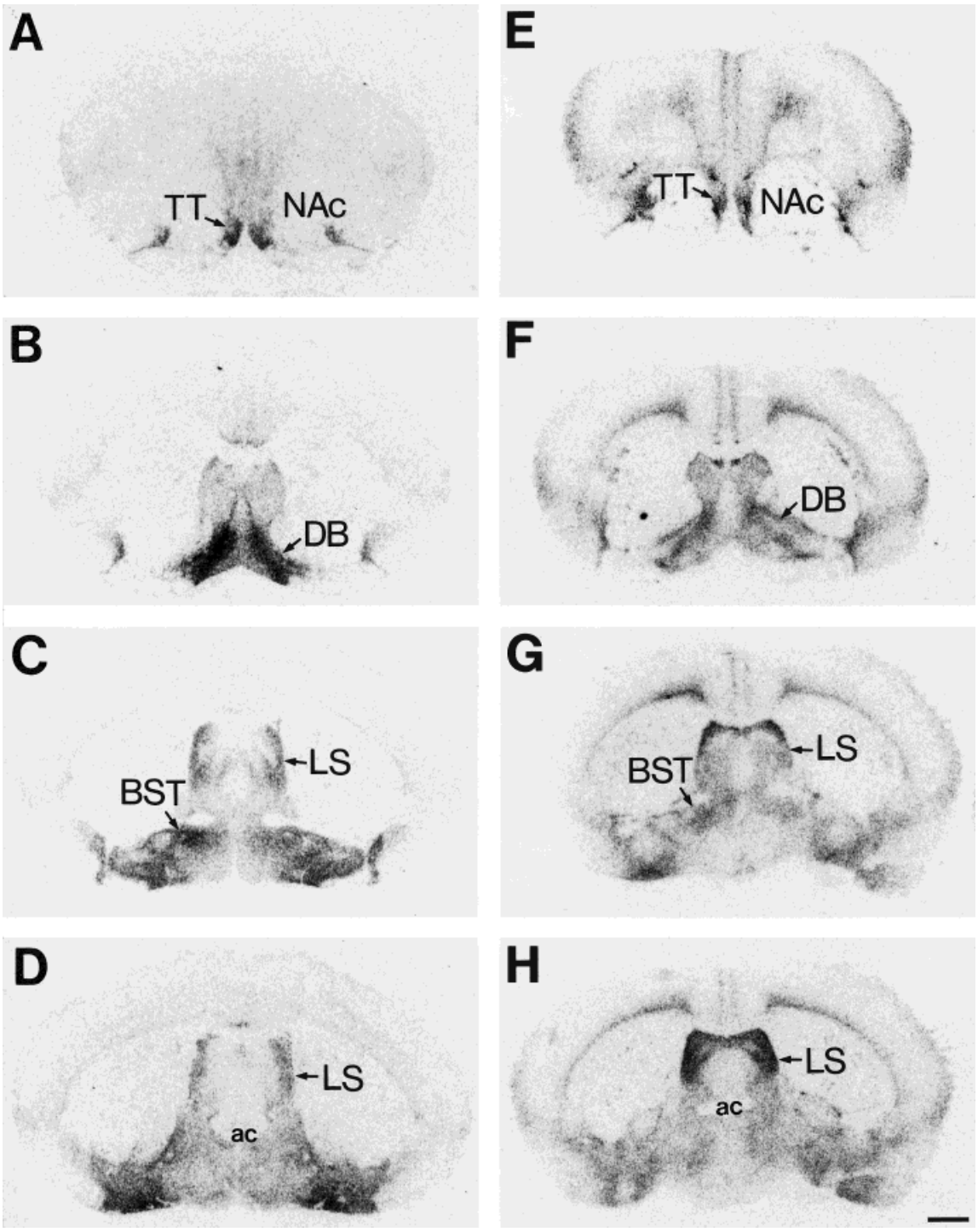

Fig. 1. Photomicrographs displaying ^{125}I-linear-vasopressin (AVP) binding in paired rostral brain sections from a prairie vole (**A–D**) and from a montane vole (**E–H**). For abbreviations, see list. Scale bar = 1 mm.

voles. This difference, with little variation during development, was sustained at weaning and into adulthood. In the LS (Figs. 4b, 5) and the dentate gyrus (Fig. 4d), both species had a low but similar level of ^{125}I-linear-AVP binding neonatally. Thereafter, the binding increased continuously and reached the adult level at weaning in one species, remaining unchanged or slightly increased in the other species. AVP receptor binding in the medial nucleus of the AMYG (Fig. 4f) and the laterodorsal hypothalamus (Fig. 7b) was equal in both species neonatally and was followed by an increase in both species, with one at a slightly higher level than the other.

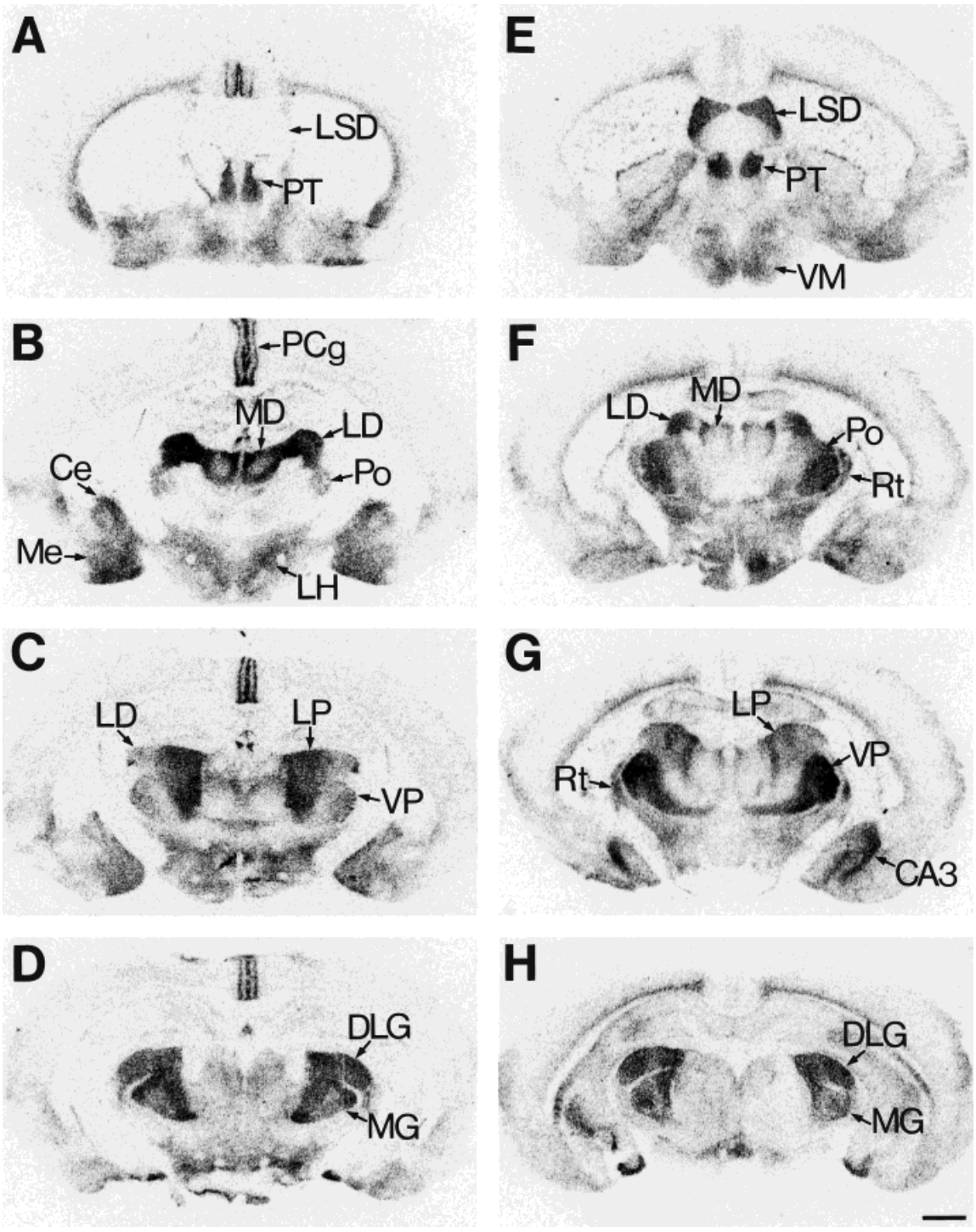

Fig. 2. Photomicrographs displaying ^{125}I-linear-AVP binding in paired caudal brain sections from a prairie vole (**A–D**) and from a montane vole (**E–H**). For abbreviations, see list. Scale bar = 1 mm.

A pattern of transient expression of ^{125}I-linear-AVP binding was found in the cortex and in the ventromedial hypothalamus. Montane voles showed intense binding in the frontoparietal cortex (Figs. 6, 7c) and in the ventromedial hypothalamus (Fig. 7a) in the first 2 weeks, with a subsequent decline to the adult level at weaning, whereas prairie voles did not show significant changes in binding during development. The opposite pattern was found in the cingulate cortex (Figs. 6, 7d), in which prairie voles showed a rapid increase that was followed by a decrease in ^{125}I-linear-AVP binding, whereas binding remained unchanged in montane voles.

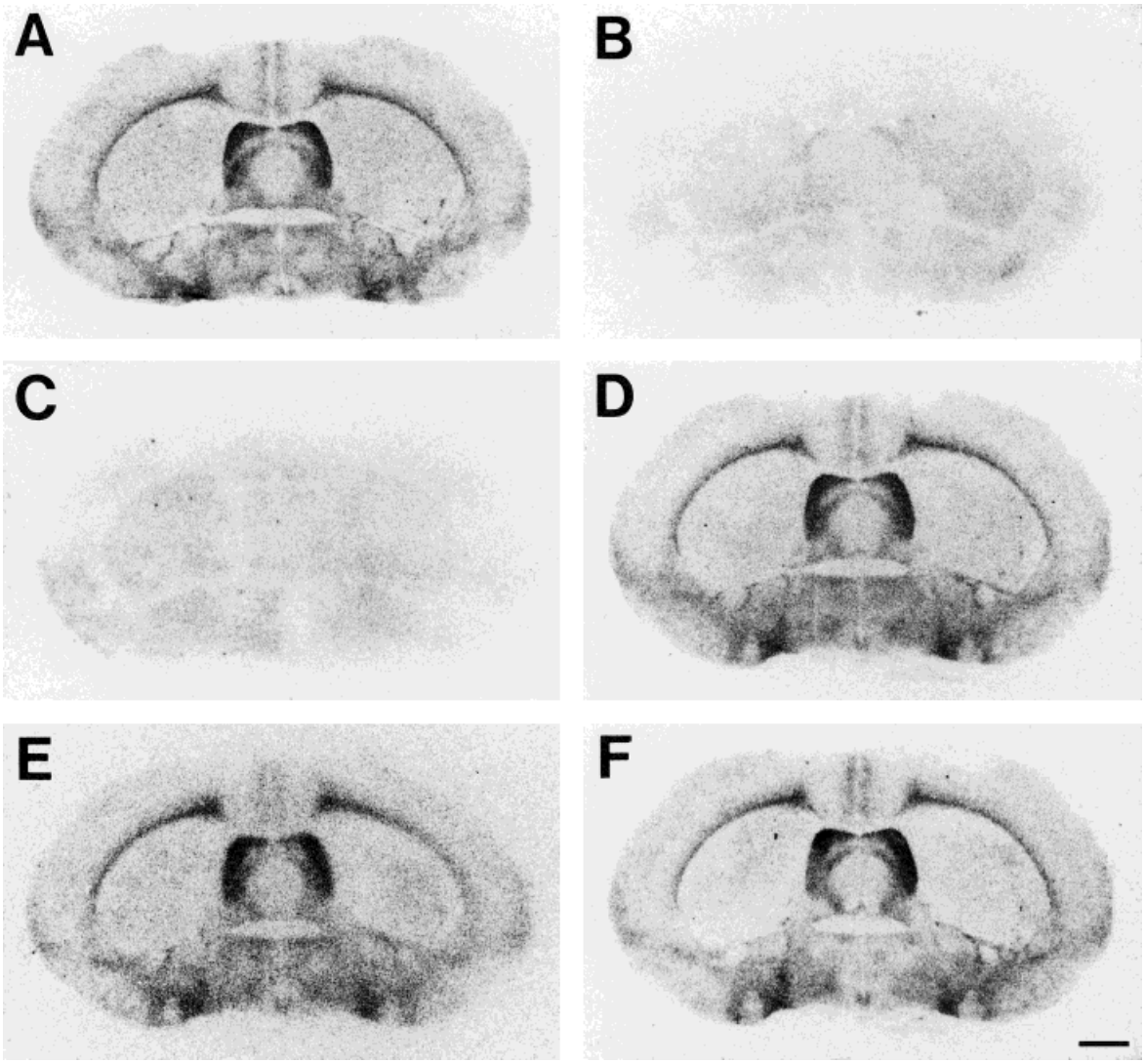

Fig. 3. Photomicrographs displaying ^{125}I-linear-AVP binding in the lateral septum (LS) in consecutive sections from an adult montane vole. Sections were incubated with ^{125}I-linear-AVP ligand (**A**), the ligand with 10^{-6} M of AVP (**B**), the V_{1a} antagonist (**C**), the V_2 antagonist (**D**), the oxytocin antagonist (**E**), or oxytocin (**F**). Scale bar = 1 mm.

DISCUSSION

In the present study, by using ^{125}I-linear-AVP, we examined the postnatal development of V_{1a} receptors in the forebrains of prairie and montane voles. Our data revealed a species-specific ontogenetic pattern of AVP receptor binding in voles, demonstrating that species differences can be detected even on the first postnatal day.

Species differences in AVP receptor binding in adults

Extensive ^{125}I-linear-AVP binding was found in the forebrain in both prairie and montane voles. In addition to the areas that were reported previously to express AVP receptors using other radiolabelled ligands (Insel et al., 1994), ^{125}I-linear-AVP bound selectively to new brain areas, such as taenia tecta, endopiriform nucleus, medial nucleus of the AMYG, periaqueductal gray, reticular thalamus, and supramammillary and medial mammillary nuclei. The selectivity of this binding essentially replicated the results found in rats (Schmidt et al., 1991; Johnson et al., 1993) and suggested that ^{125}I-linear-AVP was binding to V_{1a} receptors in both species of voles. We have recently cloned and sequenced the V_{1a} receptor in both species of voles and found that 1) these species share the same receptor, and 2) in situ hybridization with a single cRNA probe identifies a pattern of AVP receptor mRNA in each species that closely resembles the distribution of binding (Young et al., 1997). Thus, the differences that are evident in Figure 1 represent species differences in localization of a single receptor and do not result from a promiscuous ligand binding to two different receptors.

Developmental changes in AVP receptor binding

^{125}I-linear-AVP binding showed early appearance and varying expression over the course of postnatal development in both prairie and montane voles. Several general patterns were observed. First, AVP receptor binding in

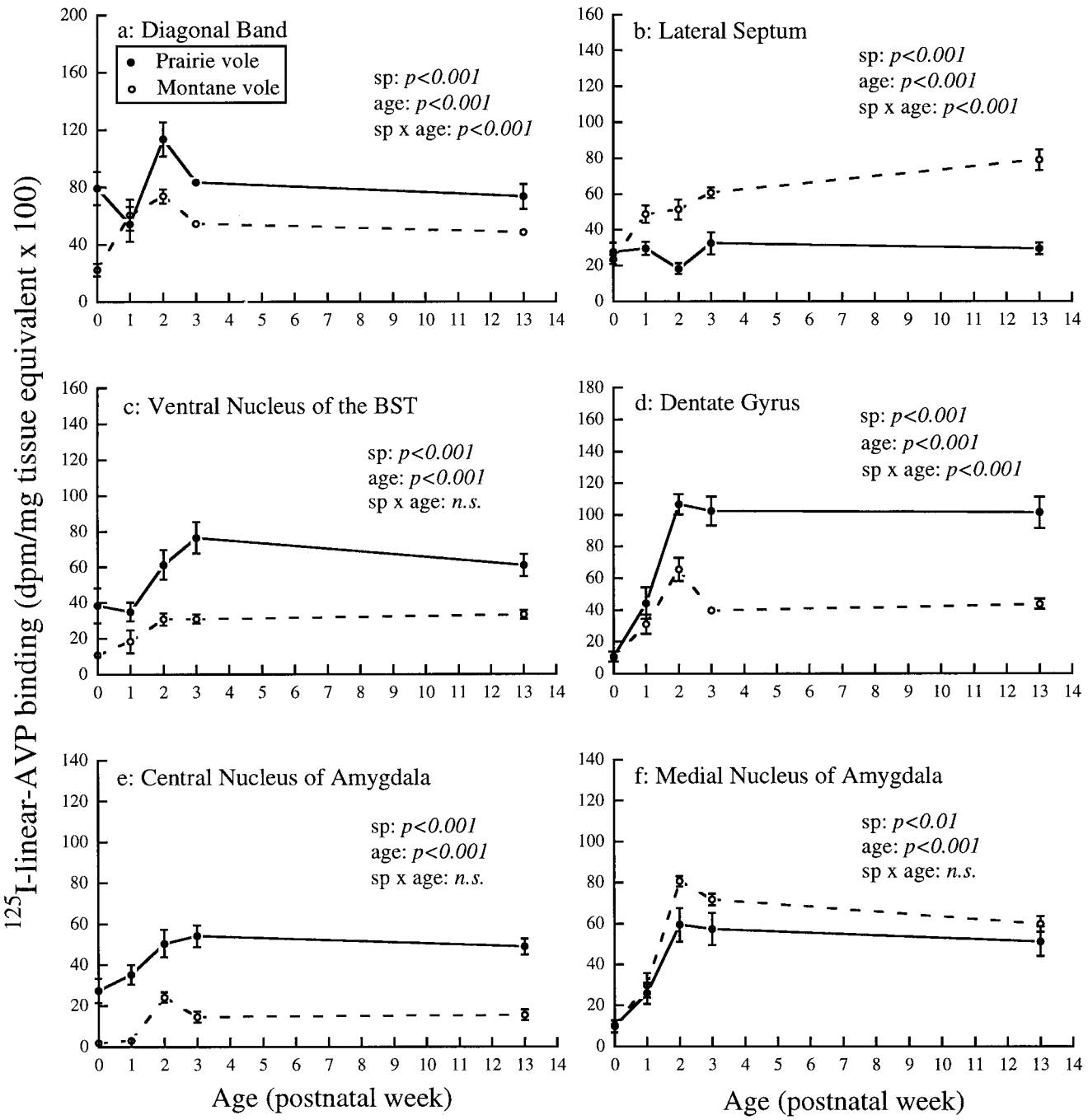

Fig. 4. Ontogeny of ^{125}I-linear-AVP binding in the diagonal band (**a**), the lateral septum (**b**), the bed nucleus of the stria terminalis (BST; **c**), the dentate gyrus (**d**), and the central (**e**) and medial (**f**) nuclei of the amygdala in prairie voles (solid circles) and in montane voles (open circles). Each point indicates a mean ± S.E.M. Analysis of variance (ANOVA) results show significant species (sp), age, or species by age (sp × age) differences. n.s., Not statistically different.

some areas emerged neonatally and showed little change during development. For example, in the LS of prairie voles and in the cingulate cortex of montane voles, ^{125}I-linear-AVP binding appeared on the day of birth and was sustained with little change into adulthood. In other brain areas, however, ^{125}I-linear-AVP binding showed a gradual increase during development and reached adult levels by weaning (3 weeks of age). This pattern was found in the AMYG and the BST in both species. The third pattern of development, which was noted in the frontoparietal cortex in montane voles and in the cingulate cortex in prairie voles, was that AVP receptors showed transient, intense labeling during early development with a subsequent decline to the low level seen in the adult brain. Whatever the patterns, differences in the AVP receptor binding in the brain between infants and adults indicate that AVP functions in developing animals may be quite different from that in adult animals (Petracca et al., 1986).

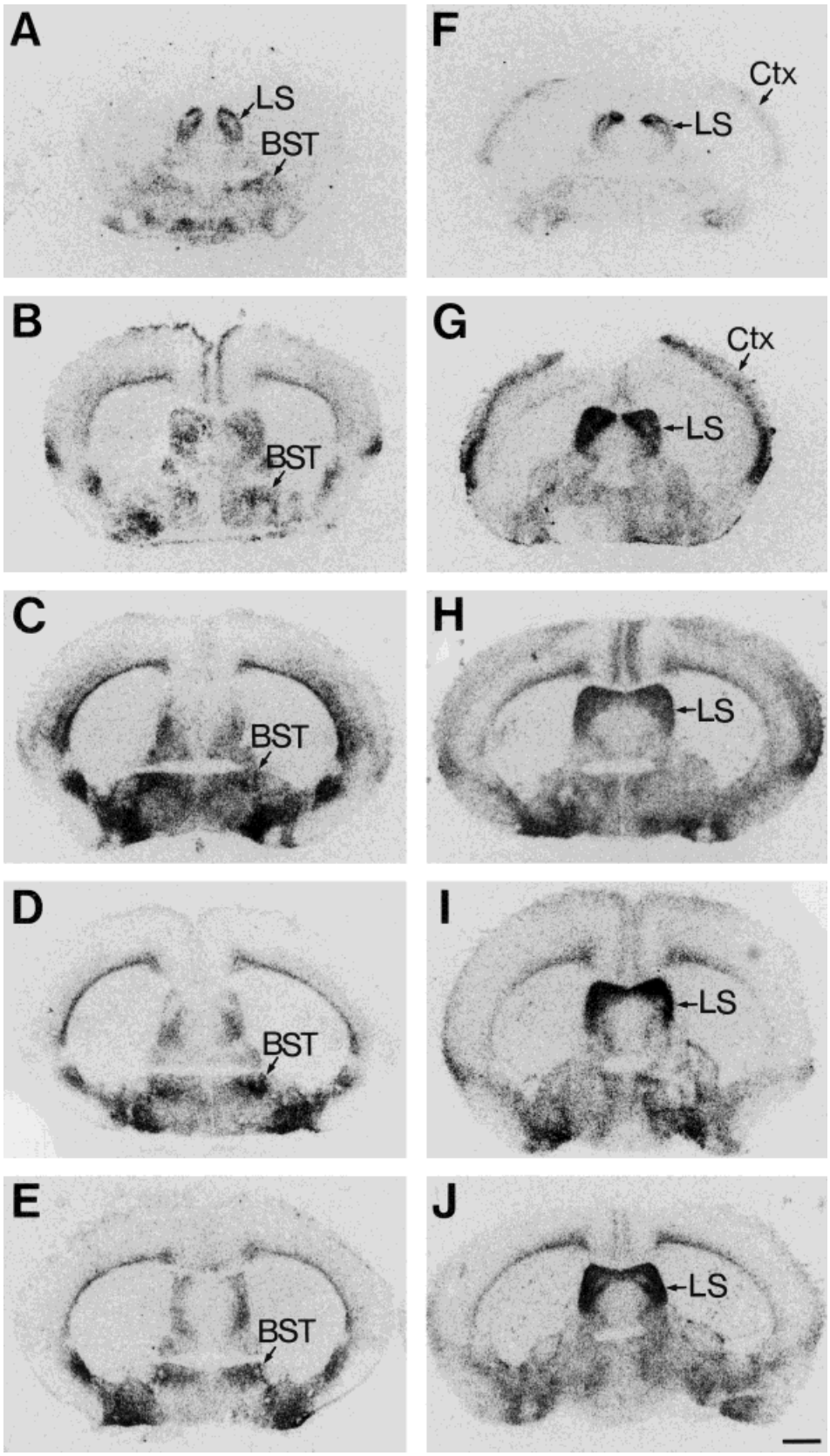

Fig. 5. Photomicrographs displaying ^{125}I-linear-AVP binding in the LS, the BST, and the frontoparietal cortex (Ctx) in prairie voles (**A–E**) and in montane voles (**F–J**) on the day of birth (A,F) and at 1 week (B,G), 2 weeks (C,H), 3 weeks (D,I), and 3 months of age (E,J). Scale bar = 1 mm.

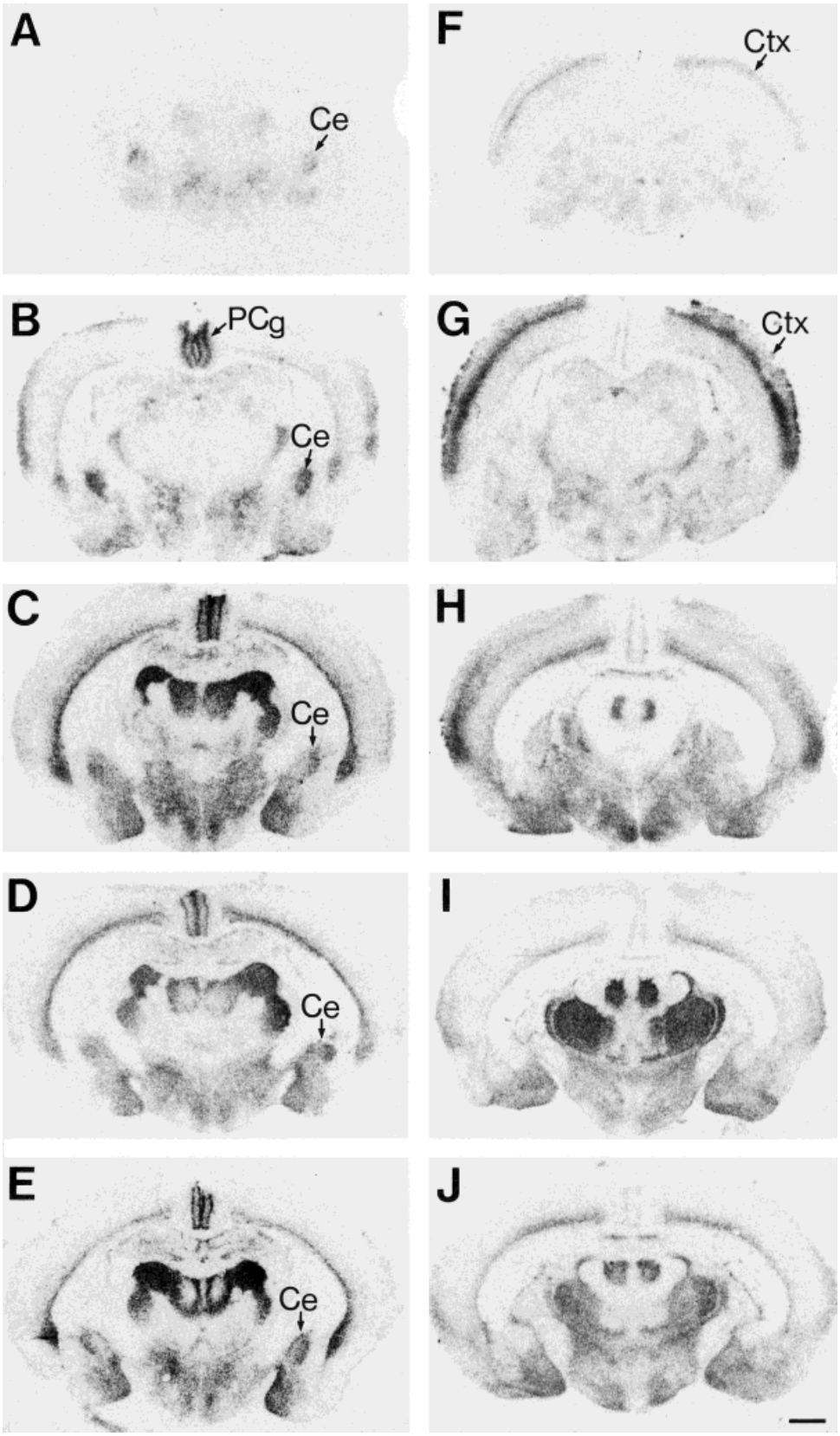

Fig. 6. Photomicrographs displaying ^{125}I-linear-AVP binding in the central nucleus of the amygdala (Ce), posterior cingulate cortex (PCg), and frontoparietal cortex (Ctx) in prairie voles (**A–E**) and in montane voles (**F–J**) on the day of birth (A,F) and at 1 week (B,G), 2 weeks (C,H), 3 weeks (D,I), and 3 months of age (E,J). Scale bar = 1 mm.

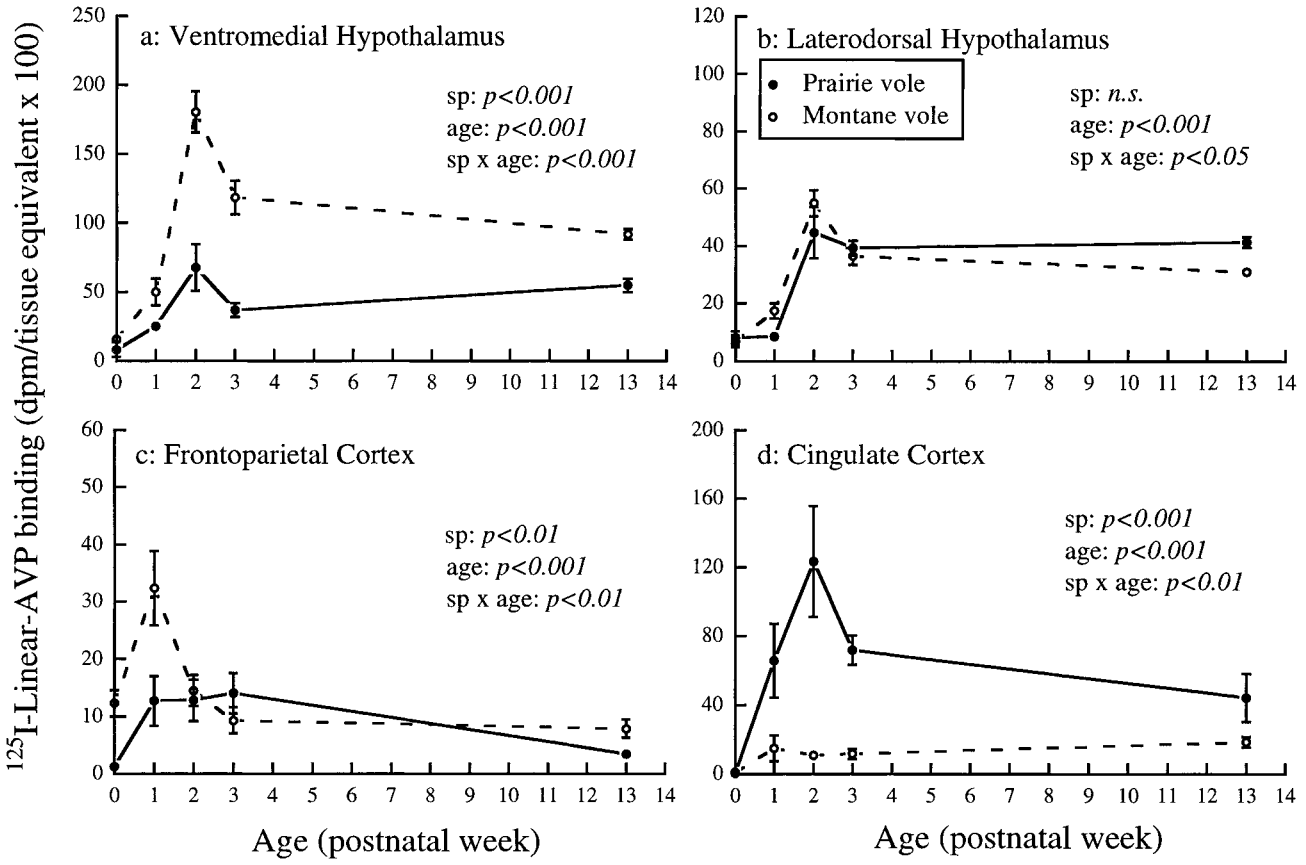

Fig. 7. Ontogeny of ^{125}I-linear-AVP binding in the ventromedial (**a**) and laterodorsal hypothalamus (**b**) and in the frontoparietal (**c**) and cingulate cortex (**d**) in prairie voles (solid circles) and in montane voles (open circles). Each point indicates a mean ± S.E.M. ANOVA results show significant species (sp), age, or species by age (sp × age) differences. n.s., Not statistically different.

What is the mechanism for these developmental changes in binding? Although it is possible that the observed differences result from developmental changes in receptor affinity, previous studies in rats have demonstrated that analogous differences in AVP receptor binding between infants and adults are due to differences in number and not to differences in affinity (Tribollet et al., 1991). At least three mechanisms could explain developmental changes in the number of receptors: 1) genesis or death of neurons that contain AVP receptors, 2) reorganization of processes that contain receptors, or 3) regional changes in V_{1a} receptor gene expression. Although our binding study could not distinguish between these mechanisms, the peak of changes in the second postnatal week is too late to be explained by the major wave of neurogenesis (which is prenatal). In addition, although an interval of dendritic outgrowth and increased synaptic density might explain the timing of the developmental peak, it seems unlikely that the absence of a peak (as in montane cingulate) can be attributed to the absence of synaptic reorganization in this brain region. Of course, it is possible that both a reorganization of connections and a developmental change in gene expression contribute to the observed ontogenetic patterns.

It is worth noting that, although changes of regional AVP receptor binding during development followed different patterns, in each case, the shift from the infant to the adult pattern took place essentially in the first 3 weeks during development. Therefore, both species of voles had the adult pattern of AVP receptor binding at weaning, which is in accordance with the finding in rats (Snijdewint et al., 1989; Tribollet et al., 1991). It was found that the adult pattern of AVP receptor binding at weaning was not subject to further changes in rats (Tribollet et al., 1991), which contrasts to development of their OT receptors, where a second change takes place at puberty (Shapiro and Insel, 1989; Tribollet et al., 1989). Early studies suggest that the postpubertal OT receptor expression in rats probably depends on changes in gonadal steroids, whereas AVP receptors are not affected (De Kloet et al., 1986; Insel, 1986; Tribollet et al., 1990). However, a recent study in hamsters has suggested that steroids alter AVP receptor binding in brain areas involved in the regulation of social behaviors (Johnson et al., 1995). In prairie voles, mating increases circulating testosterone (Gaines et al., 1985; Wang et al., 1994b). However, whether the AVP receptor in the vole brain undergoes additional changes during reproduction needs to be studied further.

Species-specific pattern of AVP receptor development

Although both species showed varying expression of ^{125}I-linear-AVP binding in the brain, prairie and montane

voles differed in the pattern of regional receptor binding during development. For example, in the DB, BST, and central nucleus of the AMYG, prairie voles had higher ^{125}I-linear-AVP binding at birth than montane voles. This difference, despite its variations during early development, was sustained at weaning and in adulthood, indicating that species differences in ^{125}I-linear-AVP binding in these brain areas were probably determined by prenatal and/or genetic factors.

In other brain areas, species differences in ^{125}I-linear-AVP binding arose postnatally. In the LS, dentate gyrus, and ventromedial hypothalamus, both species had a low but equal amount of ^{125}I-linear-AVP binding at birth. Thereafter, the binding increased rapidly in one species but increased slowly or remained unchanged in the other. These data suggest that species differences in ^{125}I-linear-AVP binding in those areas may be derived from differences in the pattern of transcriptional regulation during postnatal development.

AVP receptors in the cortex showed a dense ^{125}I-linear-AVP binding in early development that was followed by a rapid decrease, reaching the adult level at weaning. This ontogenetic peak of cortex receptor binding has been observed in AVP (Snijdewint et al., 1989; Tribollet et al., 1991), OT (Shapiro and Insel, 1989; Tribollet et al., 1989), and other peptide receptors in rats (Quirion and Dam, 1986; Insel et al., 1988; Palacios et al., 1988). In the current study, this developmental pattern appeared to be regionally species specific, because montane voles showed a peak of ^{125}I-linear-AVP binding in the frontoparietal cortex in the first week, whereas prairie voles exhibited dense AVP receptor binding in the cingulate cortex in the second week during development.

The mechanism underlying species differences in the ontogenetic pattern of AVP receptors is still unknown. One possibility is that developmental differences are due to ontogenetic changes in transcription factors that control gene expression. The species could differ either in the 5′ flanking region of the V_{1a} receptor gene or in the concentration of transcription factors through development. We are currently sequencing the 5′ flanking region of the V_{1a} receptor gene in both species to compare promoter elements in prairie and montane voles.

CONCLUSIONS

In summary, our results indicate that prairie voles and montane voles differ in the regional pattern of AVP receptor binding as early as the first postnatal day. A previous report has demonstrated that transiently expressed receptors in the rat brain are functionally responsive to AVP (Tribollet et al., 1991). These new data suggest that not only are there remarkable species differences in the neural circuits responsive to AVP in adult voles, but, even in development, AVP may be exerting different functional effects in the two species. Along with other colleagues, we have previously demonstrated a role for AVP in complex social behaviors in voles (Winslow et al., 1993; Wang et al., 1994a) and in separation responses in rat pups (Winslow and Insel, 1993). One might speculate that the species differences in patterns of AVP receptor in development contribute to the contrasting patterns of both social organization and neural organization in adult prairie and montane voles.

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