I.J.A. Urban, J.P.H. Burbach and D. De Wied (Eds.)
Progress in Brain Research, Vol. 119

CHAPTER 5.7

Voles and vasopressin: A review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors

Zuoxin Wang[a,b,*], Larry J. Young[b], Geert J. De Vries[c], Thomas R. Insel[b,d]

[a]*Department of Psychology, Florida State University, Tallahassee, FL 32306-1270, USA*
[b]*Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, 1639 Pierce Drive, Atlanta, GA 30322, USA*
[c]*Department of Psychology, University of Massachusetts, Amherst, MA 01003, USA*
[c]*Yerkes Regional Primate Research Center, Emory University School of Medicine, Atlanta, GA 30322, USA*

Several lines of evidence have implicated the neurohypophyseal peptide, vasopressin (VP), in the mediation of complex social behaviors including affiliation, aggression, juvenile recognition and parental behavior. Recent studies in microtine rodents using cellular, molecular and behavioral approaches provide additional evidence suggesting a role for VP in the formation of pair bonding and male parental care. Monogamous and promiscuous voles differ in social behaviors such as mating-induced pair bonding, selective aggression, and male parental care. Comparative studies have demonstrated that they also differ in dynamics of VP synthesis and release associated with reproduction, in the distribution pattern and regional quantity of VP receptors, and in the promoter sequence of the V_{1a} receptor gene. In monogamous prairie voles, (*Microtus ochrogaster*), brain administration of VP induces pair bonding and male parental care whereas administration of the VP antagonist diminishes these behaviors. Together, these data suggest that VP is involved in the regulation of social behaviors in monogamous voles and differences in the brain VP system may underlie species differences in behavior and life strategy in voles.

Introduction

Beginning with de Wied's 1965 report of vasopressin (VP) effects on memory, a generation of research has demonstrated that vasopressin influences behavior and cognition via actions within the central nervous system. In addition to the effects on memory (de Wied, 1977; Dantzer et al., 1988; Bluthe et al., 1990), central VP pathways have been implicated in the regulation of body temperature (Meisenberg and Simmons, 1983; Wilkinson and Kastling, 1987), blood pressure (Pittman et al., 1982), brain development (Boer et al., 1980, 1982), and social behaviors (Ferris et al., 1984; Irvin et al., 1990; Koolhaas et al., 1990; Albers et al., 1992). Virtually all of the studies on VP's central actions have used common laboratory rodents, such as rats, mice and hamsters. Although these species account for the overwhelming majority of research subjects in behavioral neuroscience, they are highly domesticated, non-selectively social, and of limited value for studying the role of VP in certain complex behaviors such as pair bonding and male parental care. In the past 5 years there have been several reports of VP's effects on social behaviors in voles. Voles are mouse-sized rodents within the genus *Microtus*. Intense field investigation has demonstrated that some American species, such as the prairie vole (*M. ochrogaster*), are monogamous and highly paternal; whereas other closely related species, such as the montane vole (*M. montanus*), are promiscuous and non-paternal. As these species differences can also be demonstrated in laboratory-bred populations of voles, these animals provide an ideal model system for comparative studies and the monogamous species permits the study of complex social beha-

* Corresponding author. Tel.: +1 850 6445057; fax: +1 850 6447739; e-mail: zwang@psy.fsu.edu.

viors, such as pair bonding and paternal care. In this review, we will summarize recent findings from studies of central VP pathways and their importance for the regulation of social behavior in voles.

The microtine model

About 3% of mammals are considered monogamous, meaning that they share a nest and territory, form pair bonds, attack intruders and show high levels of paternal care (Kleiman, 1977; Dewsbury, 1987). In the laboratory and the field, prairie voles and pine voles (*M. pinetorum*) demonstrate each of these features (Table 1) (Getz et al., 1981; FitzGerald and Madison, 1983; McGuire and Novak, 1984; Carter et al., 1986; Oliveras and Novak, 1986; Shapiro and Dewsbury, 1990; Wang and Insel, 1996). In contrast, montane voles and meadow voles (*M. pennsylvanicus*) are non-monogamous. Their social structure, usually considered promiscuous, lacks pair bonding or nest sharing between males and females during the breeding season (Jannett, 1980, 1982; Madison, 1980; Gruder-Adams and Getz, 1985; Shapiro and Dewsbury, 1990; Insel et al., 1995). The female is the lone provider of parental care (McGuire and Novak, 1984, 1986). In contrast to the monogamous species that inhabit multigenerational, communal burrows, montane or meadow voles are generally found in isolated burrows (Madison, 1980; Jannett, 1982). Species differences in social behavior can be observed even in neonatal voles: infant prairie voles respond to social separation as a stressor whereas infant montane voles show no evident behavioral or endocrine response to social separation (Shapiro and Insel, 1990).

In all of these vole species, mating is a prolonged affair with multiple copulatory bouts over a 24 h period. The behavioral consequences of mating are markedly different in monogamous and non-monogamous voles. In the prairie vole, for instance, 24 h of mating establishes a pair bond between a male and a female, evident by the formation of a preference for the mate versus a conspecific stranger and the emergence of aggression towards intruders into the nest (Carter et al., 1986; Shapiro and Dewsbury, 1990; Carter and Getz, 1993; Winslow et al., 1993; Insel et al., 1995). The partner preference and nest guarding persist for at least 2 weeks even in the absence of further exposure to the mate (Winslow et al., 1993; Insel et al., 1995). After parturition, both parents display intense parental care (McGuire and Novak, 1984; Oliveras and Novak, 1986). On the other hand, non-monogamous voles, such as montane or meadow voles, show little interest in social contact, mating does not induce a pair bond and aggression, and males display little if any parental care (McGuire and Novak, 1986; Oliveras and Novak, 1986; Shapiro and Dewsbury, 1990; Insel et al., 1995).

At least three aspects make voles particularly useful for the study of VP. First, the profound differences in pair bond and parental behavior between vole species provide an intriguing natural experiment for studying neuroanatomic features (e.g. VP pathways) associated with evolved patterns of social behaviors. Second, the development of social behaviors triggered by reproductive events such as mating or parturition in the monogamous species, permit the study of neural mechanisms of pair bonding. Studies in the prairie vole might indicate if reproductive behavior influences VP or if VP influences pair bonding and parental care. Finally, sexual dimorphism tends to be mini-

Table 1
Social organization and behaviors in voles

	Praire (*M. ochrogaster*)	Pine (*M. pinetorum*)	Montane (*M. montanus*)	Meadow (*M. pennsylvanicus*)
Life strategy	Monogamous	Monogamous	Promiscuous	Promiscuous
Pair bond	Yes	?	No	?
Nest sharing	Yes	Yes	No	No
Parental	Biparental	Biparental	Maternal	Maternal

mal in monogamous species (Emlen and Oring, 1977; Shapiro et al., 1991). Vasopressin has been previously shown to be highly sexually dimorphic in the rat brain (De Vries, 1990). Study of monogamous voles, therefore, might indicate if sexual dimorphisms persist in the brains of rodents when males and females perform similar social roles.

Diversity of central vasopressin systems in voles

We have used the comparative approach to determine if voles with different social organization and behavior show differences in VP cells or projections, VP receptor distribution or VP receptor gene sequence. These studies asked (a) how do monogamous species differ from non-monogamous species? and (b) how do males and females differ within the monogamous species?

Vasopressin cells and fibers

Central VP immunoreactivity and mRNA expression have been examined in a variety of vole species (Bamshad et al., 1993; Wang et al., 1994b, 1996; Wang, 1995). VP immunoreactive (VP-ir) or VP mRNA-labeled cells are found in dense clusters in the paraventricular (PVN), supraoptic (SON) and, to a lesser extent, in the suprachiasmatic (SCN) nucleus of the hypothalamus. Scattered VP-ir or VP mRNA-labeled cells are found in extrahypothalamic areas such as the bed nucleus of the stria terminalis (BST) and medial nucleus of the amygdala (MA). VP-ir fibers are found in several brain areas such as the lateral septum (LS), lateral habenular nucleus (LH), diagonal band (DB), medial preoptic area (MPO), BST, PVN and the MA. The morphology and distribution pattern of these VP cells and fibers are not only similar among voles that have different social organization and behaviors, but also resemble those found in other species of rodents (Buijs et al., 1978; De Vries et al., 1985; Mayes et al., 1988; Hermes et al., 1990; Bittman et al., 1991).

A sexually dimorphic pattern is evident for VP pathways in voles. Contrary to prediction, marked sexual dimorphisms are evident in both monogamous and non-monogamous voles. Across four species (prairie, pine, montane and meadow), males have more VP-ir or VP mRNA-labeled cells in the BST, and a higher density of VP-ir fibers in the LS and LH than females (Fig. 1; Bamshad et al., 1993; Wang et al., 1994b, 1996). This sexually dimorphic VP pathway is steroid dependent: castration reduces the number of VP-ir cells in the BST and MA as well as the density of VP-ir fibers in the LS and LH, whereas testosterone replacement restores the VP-ir staining of castrated males to the level of intact male voles (Fig. 2; Wang and De Vries, 1993). This gender dimorphism and steroid dependence has been previously reported in several species of rodents such as rats, mice, hamsters and gerbils (van Leeuwen et al., 1985; Mayes et al., 1988; Hermes et al., 1990; Bittman et al., 1991; Crenshaw et al., 1992). Castration and testosterone treatment do not induce significant changes in VP expression in the hypothalamic nuclei (PVN, SON and SCN) in voles (Wang and De Vries, 1993; Wang, Young and Insel, unpublished data) nor in other species of rodents (Crowley and Amico, 1993). In spite of marked differences in behavior, the morphology, distribution, and characteristics suggest that VP synthesizing cells and fibers are similar across voles and, in general, voles resemble pathways reported in other species of rodents.

Vasopressin receptor distribution

The actions of VP are mediated by three subtypes of membrane-bound receptors, namely V_{1a}, V_{1b} and V_2 receptors, with V_{1a} predominating in the central nervous system (Jard, 1983; Van Leeuwen et al., 1987; Barberis and Tribollet, 1996). Voles with different social organization and behavior differ in their brain VP receptor distribution. Studies using tritiated (^{3}H-VP) and iodinated VP ligands (^{125}I-sarc-VP) with in vitro receptor autoradiography have demonstrated that monogamous prairie and pine voles show a similar pattern of VP receptor binding, with little overlap with the receptor distribution observed in promiscuous montane and meadow voles (Insel et al., 1994). In addition, male and female voles show a similar distribution pattern of VP receptor binding within each species. These data suggest that species differences in the distribution pattern of central VP receptors appear to be

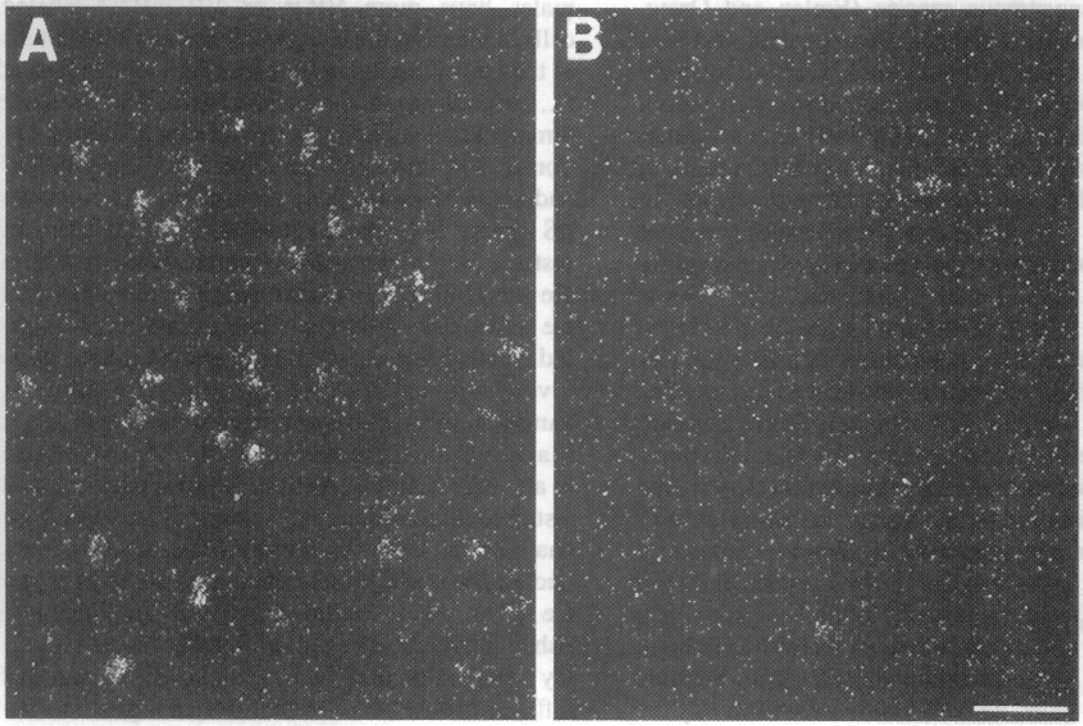


Fig. 1. Photomicrographs of dark-field-illuminated sections displaying cells labeled for VP mRNA in the bed nucleus of the stria terminalis in male (A) and female (B) prairie voles. Scale bar, 50 mm (adapted from Wang et al., 1994b).

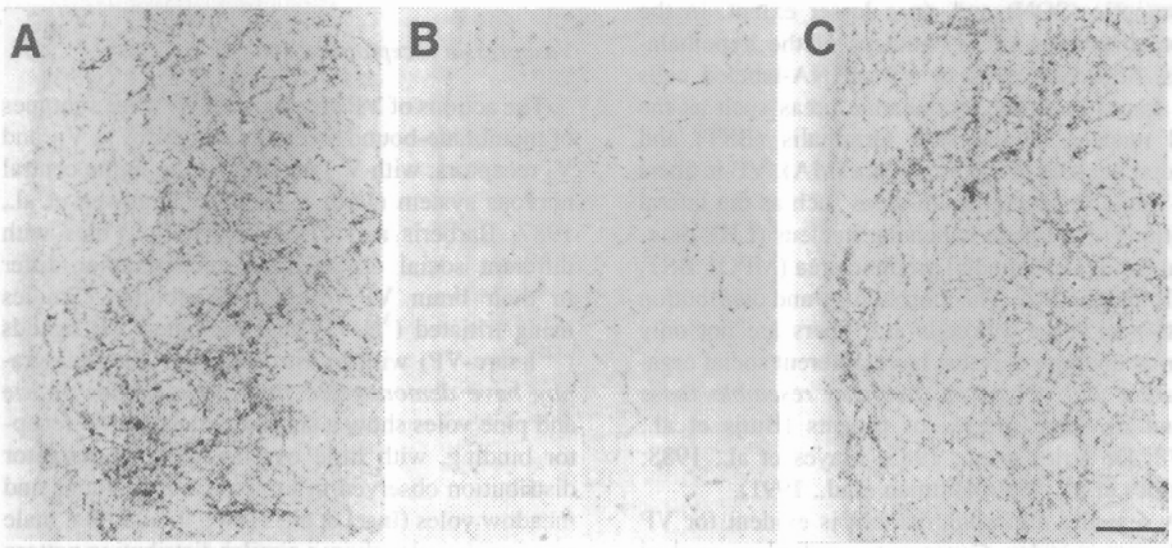


Fig. 2. Photomicrographs displaying VP-ir fiber plexus in the lateral septum of male prairie voles that were sham-operated (A), castrated for 4 weeks (B), or castrated and implanted with testosterone (C). Scale bar, 50 mm (adapted from Wang and De Vries, 1993).

associated with the pattern of social organization in voles. In a recent study using newly developed ^{125}I-linear-VP ligand (Johnson et al., 1993; Barberis et al., 1995), VP receptor binding was identified in anatomically matched coronal sections from prairie and montane voles (Fig. 3; Wang et al., 1997). Prairie voles have a higher density of VP receptors labeled by ^{125}I-linear-VP in the diagonal band, central nucleus of the amygdala, cingulate cortex and laterodorsal thalamus than montane voles. On the other hand, montane voles have more VP receptor binding in the lateral septum, ventroposterior and reticular thalamus than prairie voles.

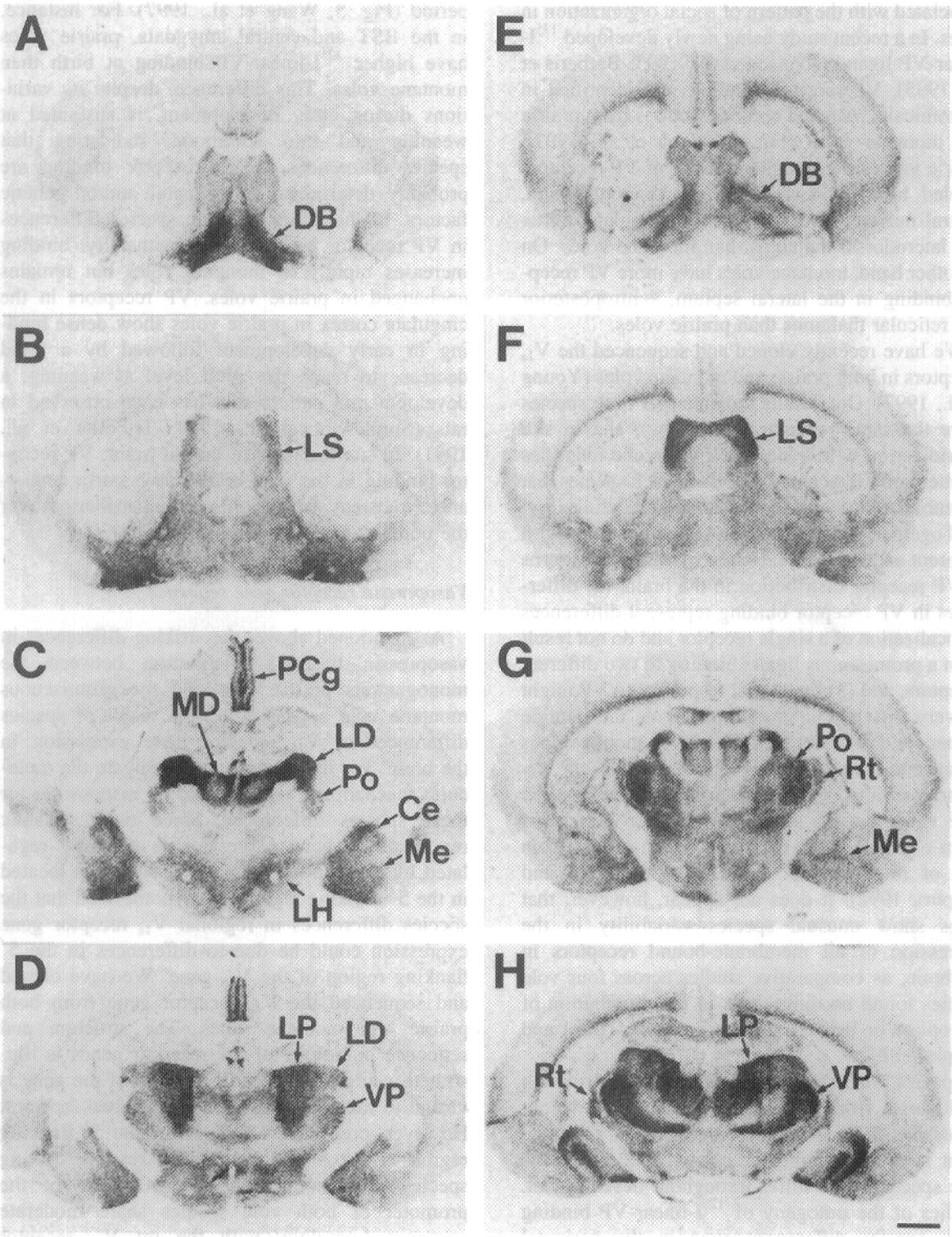
We have recently cloned and sequenced the V_{1a} receptors in both prairie and montane voles (Young et al., 1997). Our results indicate that both species share the same receptor (see below), and in situ hybridization with a single cRNA probe identifies a pattern of VP receptor mRNA in each species that resembles the distribution pattern of binding (Fig. 4). Together, these data suggest that (1) voles with different social organization have different patterns of VP receptor distribution in the brain, (2) differences in VP receptor binding represent differences in localization of a single receptor and do not result from a promiscuous ligand binding to two different receptors, and (3) functional responses to VP might be very different in these species as the peptide influences a different set of targets in monogamous versus non-monogamous voles.

In a previous study, we reported that prairie and pine voles show a similar distribution pattern of brain oxytocin (OT) receptors which differs from that of montane and meadow voles (Insel and Shapiro, 1992). It does not appear, however, that voles show unusual species variability in the expression of all membrane-bound receptors in the brain, as comparative studies across four vole species found no differences in the distribution of mu opiate or benzodiazepine receptors (Insel and Shapiro, 1992).

Species differences in receptor distribution could arise from a common pattern in development with the two species diverging at a critical point such as weaning or puberty. Alternatively, vole species could differ throughout development. Studies of the ontogeny of ^{125}I-linear-VP binding show species differences even in the perinatal period (Fig. 5; Wang et al., 1997). For instance, in the BST and central amygdala, prairie voles have higher ^{125}I-linear-VP binding at birth than montane voles. This difference, despite its variations during early development, is sustained at weaning and into adulthood, indicating that species differences in VP receptor binding are probably determined by prenatal and/or genetic factors. In the lateral septum, species differences in VP receptor binding arise postnatally: binding increases rapidly in montane voles but remains unchanged in prairie voles. VP receptors in the cingulate cortex in prairie voles show dense binding in early development followed by a rapid decrease to reach the adult level at weaning: a developmental pattern that has been observed in rats (Snijdewint et al., 1989; Tribollet et al., 1991). In summary, as in the rat brain, VP receptor binding in the vole brain shows early appearance, transient expression, and redistribution over the course of postnatal development.

Vasopressin receptor gene sequence

As mentioned above, the striking differences in vasopressin receptor distribution between the monogamous prairie vole and the promiscuous montane vole appear to be the result of species differences in V_{1a} receptor gene expression in the brain. We have begun to investigate the molecular mechanisms which could be responsible for these species differences. Since tissue specific regulation of gene expression is typically regulated by specific *cis* regulatory sequences located in the 5′ flanking region, we hypothesized that the species differences in regional V_{1a} receptor gene expression could be due to differences in the 5′ flanking region of the V_{1a} gene. We have cloned and sequenced the V_{1a} receptor gene from both prairie and montane voles. The structure and sequence homology of the receptor genes is illustrated in Fig. 6. The coding region of the gene is virtually identical between these two species. However, preliminary analysis of the 5′ flanking region of the V_{1a} receptor gene reveals striking species differences. The first 1000 bp of the promoter of both vole species share moderate sequence homology with the rat V_{1a} receptor


A
DB
E
DB
B
LS
F
LS
C
PCg
MD
LD
Po
Ce
Me
LH
G
Po
Rt
Me
D
LP
LD
VP
H
LP
Rt
VP

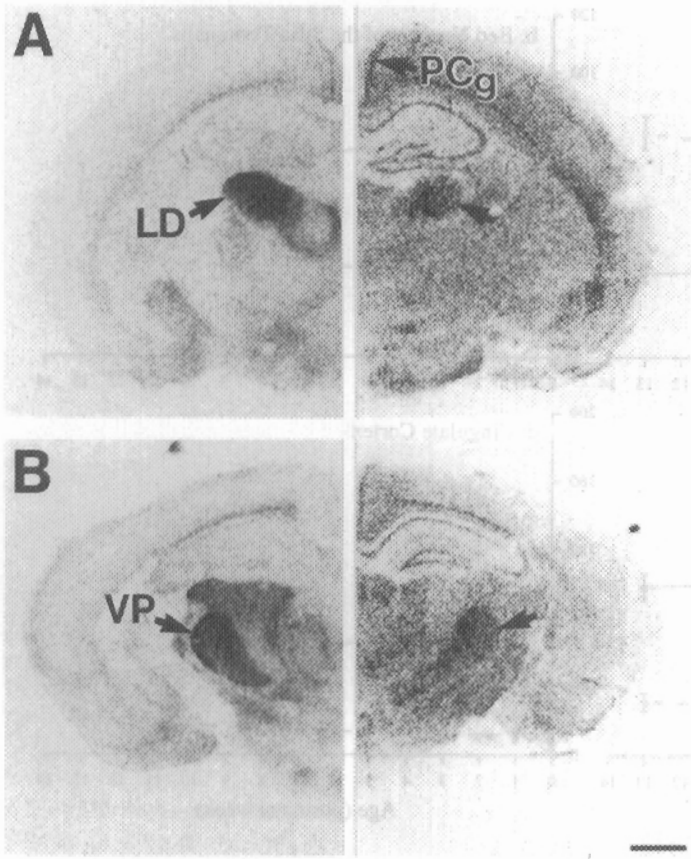


Fig. 4. Photomicrographs displaying VP receptor binding (left) and mRNA labeling (right) on the brain section of a prairie (A) and a montane vole (B). Scale bar, 1 mm (adapted from Young et al., 1997).

gene (Murasawa et al., 1995), and high homology with each other. However, a sudden complete loss of sequence homology is found beyond 1000 bp. Comparison of this region with the rat V_{1a} receptor gene reveals significant homology between the rat sequence and the montane vole sequence. This sequence homology between the montane vole and rat might indicate that the montane vole V_{1a} receptor gene is the ancestral form, while the prairie vole gene is the derived form. This hypothesis is also supported by the similarity in V_{1a} receptor binding pattern in the rat and montane vole. The sequence data suggest that the promoter of the prairie vole V_{1a} receptor gene, or that of a recent ancestor, has been modified, most likely due to translocation or an insertional event. This event may be related to the species difference in V_{1a} receptor gene expression, and potentially to the evolution of social behavior in the prairie vole. We are currently investigating this hypothesis by analyzing the promoters of other vole

Fig. 3. Photomicrographs displaying ^{125}I-linear-VP binding in paired coronal sections from a prairie vole (A–D) and a montane vole (E–H). Ce, central amygdala; DB, diagonal band; LD, laterodorsal thalamus; LH, laterodorsal hypothalamus; LP, lateroposterior thalamus; LS, lateral septum; MD, mediodorsal thalamus; Me, medial amygdala; PCg, cingulate cortex; Po, posterior thalamus; Rt, reticular thalamus; VP, ventroposterior thalamus. Scale bar, 1 mm (adapted from Wang et al., 1997).

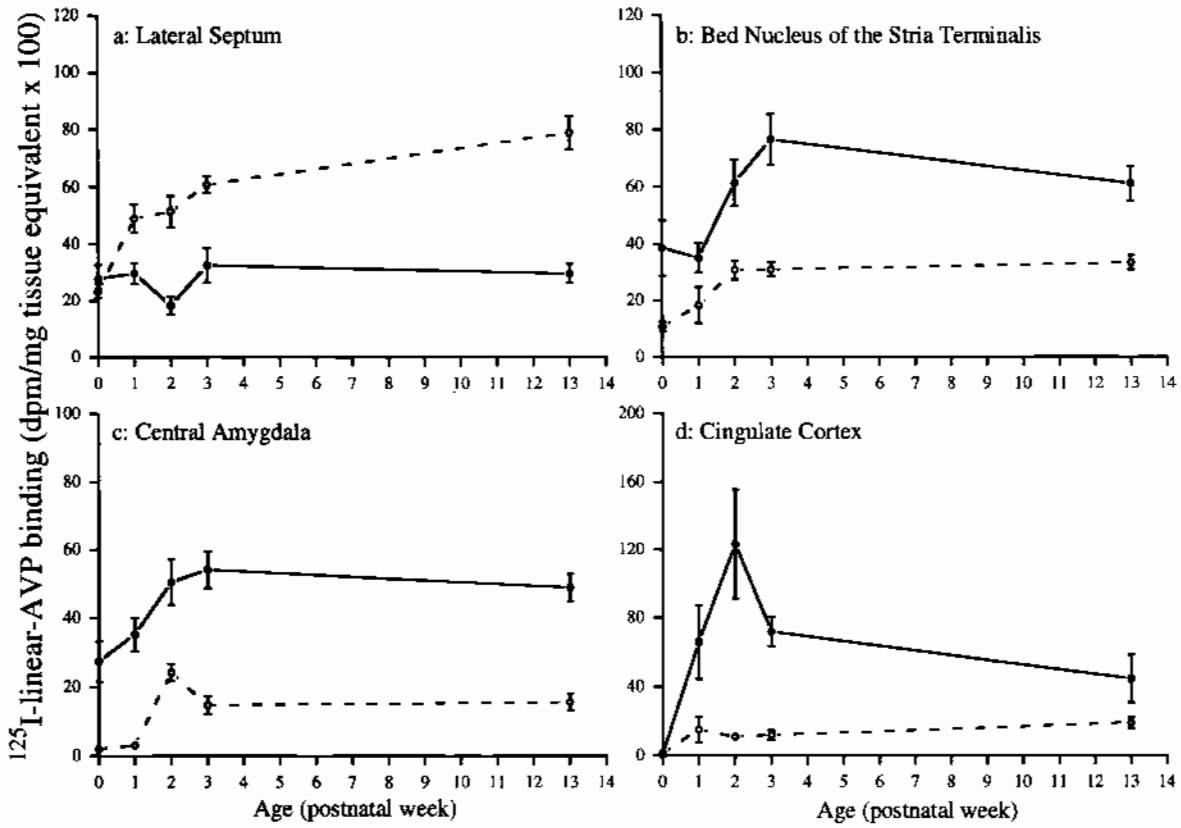


Fig. 5. Ontogeny of ^{125}I-linear-VP binding in the lateral septum (a), bed nucleus of the stria terminalis (b), central nucleus of the amygdala (c) and cingulate cortex (d) in prairie voles (●) and montane voles (○). Each point indicates a mean ± SEM from five to six animals (adapted from Wang et al., 1997).

species with similar differences in receptor distribution and social behavior.

Changes of central vasopressin associated with reproductive behavior

Voles with different social organization and behaviors show differences not only in their VP receptor distribution, but also in the dynamics of central VP release and gene expression. We have investigated changes of central VP activity during mating and reproduction in both monogamous and promiscuous voles.

Mating-induced changes in vasopressin activity

Mating induces a behavioral transformation in prairie voles. After 24 h of mating, male and female prairie voles exhibit affiliative behavior only towards their mates, and males attack conspecific strangers (Shapiro and Dewsbury, 1990; Winslow et al., 1993; Insel et al., 1995). Both affiliation and aggression appear to be dependent on mating as cohabitation with a female without mating does not induce such behaviors (Winslow et al., 1993; Insel et al., 1995). Furthermore, the emergence of affiliation and aggression in prairie voles appears to represent the development of a pair bond as non-monogamous voles fail to show these behaviors after mating (Shapiro and Dewsbury, 1990; Insel et al., 1995).

In addition to effects on behavior, mating also induces changes in extra-hypothalamic VP activity in the vole brain in a sex- and species-dependent

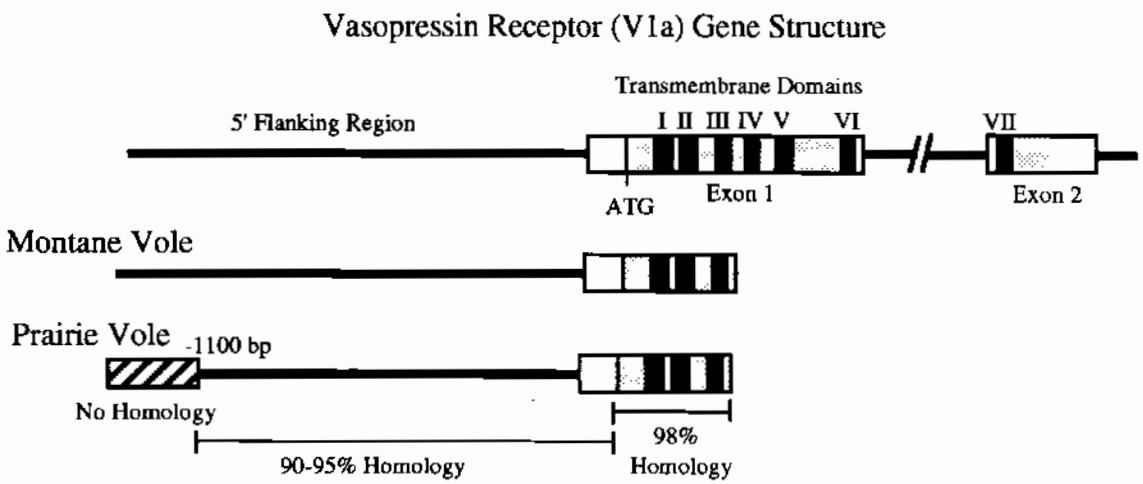


Fig. 6. The sequence homology of the 5′ end of montane vole and prairie vole V_{1a} receptor clones is illustrated below the a schematic of the full length V_{1a} receptor gene. The coding sequences share 98% homology while the first 1100 bp of the 5′ flanking region share 90–95% sequence homology between the vole species. At −1100 bp the sequence homology abruptly ends (stripped box). The montane vole sequence in this region continues to show homology with the rat gene sequence suggesting that the prairie vole sequence has been modified.

manner. After 3 days of mating and cohabitation with a female, male prairie voles have a reduced density of VP-ir fibers in the lateral septum and an enhanced level of VP mRNA expression in the BST relative to sexually naive males (Fig. 7; Bamshad et al., 1994; Wang et al., 1994b). The enhanced VP mRNA expression in the BST is associated with increased plasma testosterone (Wang et al., 1994b). In rodents, VP cells in the BST project to the lateral septum (De Vries and Buijs, 1983; De

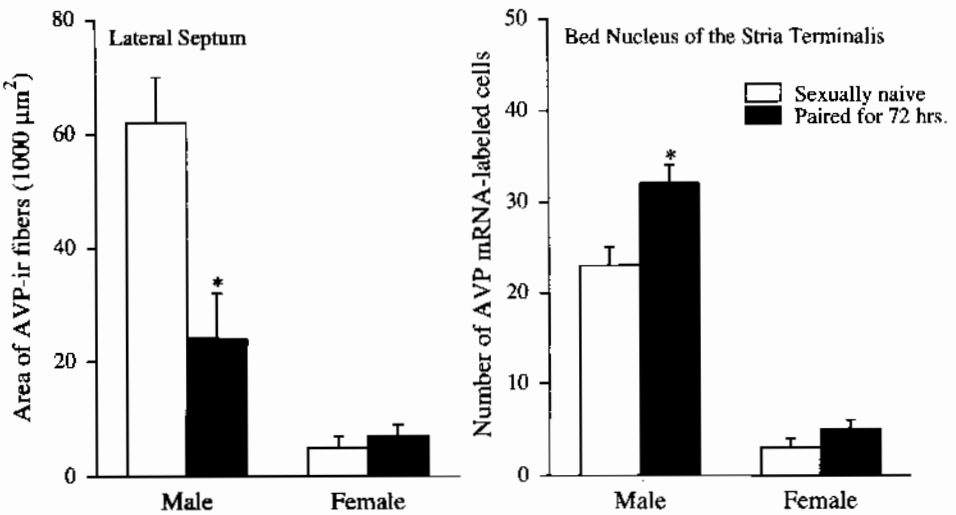


Fig. 7. Effects of 3 days mating and cohabitation on the density of VP-ir fibers in the lateral septum (left) and the number of VP mRNA-labeled cells in the bed nucleus of the stria terminalis (right) in male and female prairie voles. Scale bars, mean ± SEM; $^*P < 0.05$ (adapted from Bamshad et al., 1994 and Wang et al., 1994b).

Vries et al., 1985). Therefore, decreased VP-ir staining in terminals along with increased VP synthesis in the cell bodies probably suggests that mating induces septal VP release in male prairie voles. Such mating effects on central VP activity are not found in female prairie voles nor are they found in either sex of non-monogamous meadow voles, suggesting that mating-induced septal VP release is male-specific and restricted to the monogamous vole (Bamshad et al., 1994; Wang et al., 1994b).

In a recent experiment, we found that mating did not alter VP mRNA expression in the hypothalamic nuclei (PVN and SON). In addition, we could not detect a mating-induced change in brain VP receptor binding in either male or female prairie voles (Wang, Young and Insel, unpublished data). Taken together, these data suggest that in male prairie voles, mating induces hormonal changes which, in turn, trigger central VP release in a specific brain area (e.g. the lateral septum). The released VP may play a role in the regulation of social behaviors such as partner preference and aggression in prairie voles, as it does in social recognition and aggression in other rodents (Le Moal et al., 1987; Dantzer et al., 1988; Compaan et al., 1993). That mating-increased testosterone has no effects on VP mRNA expression in the PVN and SON in male prairie voles is in agreement with the finding in rats, in which castration and testosterone treatment do not induce changes in VP mRNA expression in these nuclei (Crowley and Amico, 1993). In non-monogamous voles, the lack of mating-induced central VP release (testosterone was not increased by mating in non-monogamous meadow voles, (Wang et al., 1994b)) as well as different central target sites of VP actions may at least partially account for the absence of mating-induced changes in their social behaviors.

Changes associated with parturition

Species differences in parental behavior are also associated with differences in extra-hypothalamic VP activity. With immunocytochemical staining, parental male prairie voles (at postpartum day 6) have a significantly reduced density of VP-ir fibers in the lateral septum and lateral habenular nucleus relative to their sexually naive counterparts (Fig. 8; Bamshad et al., 1993). In contrast, meadow vole fathers at postpartum day 6 do not show parental behavior to their young and have a level of VP-ir staining in both brain areas equivalent to that of sexually naive males (Fig. 8). No changes in VP-ir staining are found in females of either species. Although it is not yet clear that parental and sexu-

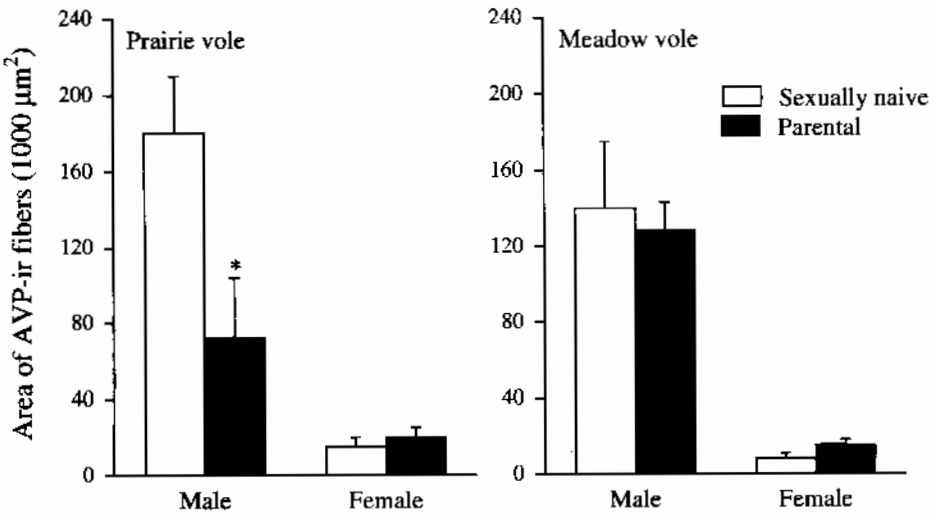


Fig. 8. Differences in the density of VP-ir fibers in the lateral septum of sexually naive (□) and parental (at postpartum day 6; ■) male and female prairie and meadow voles. Scale bars, mean ± SEM; $*P < 0.05$ (adapted from Bamshad et al., 1993).

ally naive male prairie voles have different levels of VP mRNA expression in the BST, reduced VP-ir staining in the terminal fields (in the lateral septum and lateral habenular nucleus) may reflect increased VP release. It appears, therefore, that both mating and paternal care may be associated with extra-hypothalamic VP release in monogamous male voles.

The effects of reproduction on VP activity are not limited to the sexually dimorphic pathways. Reproduction is also found to influence VP mRNA expression in the hypothalamic nuclei. Female prairie voles at postpartum day 1 and 6 have increased VP mRNA (Fig. 9) as well as OT mRNA expression in the PVN and SON relative to sexually naive females. These data resemble

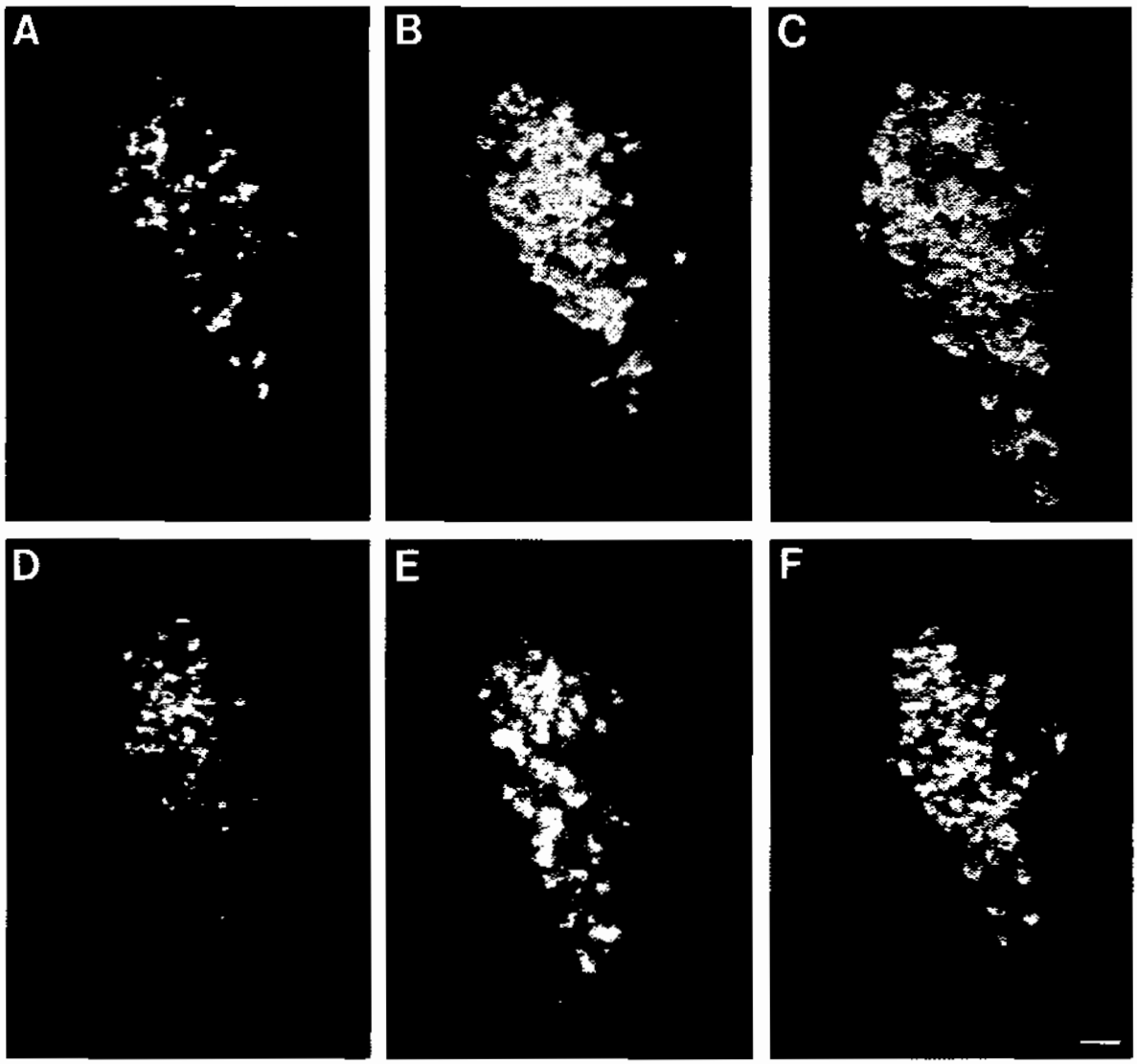


Fig. 9. Photomicrographs of dark-field-illuminated sections displaying VP mRNA-labeled cells in the paraventricular nucleus of the hypothalamus of male (A–C) and female (D–F) prairie voles. Sexually naive voles (A and D) had less VP mRNA expression in the PVN than voles at day of litter birth (postpartum day 1; B and E) or at postpartum day 6 (C and F). Scale bar, 50 mm.

results from female rats in which pregnancy, parturition and lactation are associated with a parallel elevation of VP and OT mRNA expression in the PVN and SON (van Tol et al., 1988; Zingg and Lefebvre, 1988), apparently due to sequential changes of gonadal steroids associated with pregnancy and parturition (Crowley et al., 1995; Thomas et al., 1995, 1996). Increased OT is related to uterine contraction at parturition, milk ejection in response to suckling and maternal behavior, whereas increased VP may be involved in uterine contraction and maintaining homeothermy and other autonomic events associated with female reproduction (Pedersen et al., 1982; Higuchi et al., 1986; Caldwell et al., 1987).

Interestingly, male prairie voles at postpartum day 1 and 6 also show an increase in VP mRNA expression in the PVN and SON relative to sexually naive males (Fig. 9). No postpartum effects, however, are detected in their OT mRNA expression. The reason for increased VP mRNA-labeling in the PVN and SON in male prairie voles is still unknown. While VP may influence the emergence of paternal behavior, it is also possible that paternal behavior influences VP gene expression. For instance, one aspect of parental behavior is anogenital licking of pups, including the ingestion of hypertonic urine (Baverstock and Green, 1975; Friedman and Bruno, 1976). As osmotic challenges increase hypothalamic VP mRNA levels (Zingg et al., 1986; Crowley et al., 1993), the increase in VP mRNA expression in male prairie voles may simply reflect urine ingestion (Bamshad et al., 1993). Non-monogamous montane or meadow voles may not show the increase in hypothalamic VP mRNA expression since they do not display paternal behavior.

Vasopressin regulation of social behavior

The above-mentioned neuroanatomical and neurochemical studies indicate species differences in VP systems in monogamous and non-monogamous voles. These studies provide correlational evidence but they fail to demonstrate that central VP is involved in social behavior in voles. Recent pharmacological studies provide direct evidence that central VP may be critical for social behavior in monogamous voles.

Involvement of central vasopressin in pair bonding

If mating facilitates pair bond formation and VP is released with mating, does VP influence the development of a pair bond? To answer this question, two aspects of pair bonding, mating-induced aggression and partner preference formation, have been studied in prairie voles (Winslow et al., 1993). Males receiving central injections of CSF or an OT antagonist exhibit aggression after 24 h of mating. However, males that receive an injection of a V_{1a} antagonist, ($d(CH_2)_5$[Tyr(Me)]VP), do not show mating-induced aggression (Fig. 10). This effect on aggression cannot be attributed to effects on mating, as sexual behavior is not influenced by the V_{1a} antagonist. In addition, the V_{1a} antagonist does not seem to be anti-aggressive per se because breeder males with established aggression show no decrease in aggression 1 or 24 h after receiving the V_{1a} antagonist injection (Winslow et al., 1993). These data suggest that the VP antagonist blocks the transition to aggression, not its expression in male prairie voles.

As noted above, following mating, male prairie

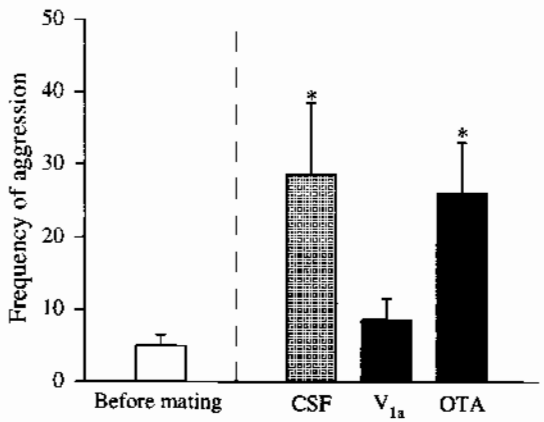


Fig. 10. Differences in male aggression after 24 h mating. Males receiving injections of a V_{1a} antagonist (5 ng) did not show mating-induced aggression whereas males receiving injections of either CSF or OTA (5 ng) showed mating-induced aggression. Scale bars, mean ± SEM: $^*P < 0.05$ (adapted from Winslow et al., 1993).

voles demonstrate a partner preference, an early manifestation of pair bonding. Males receiving an infusion of CSF or OT antagonist exhibit mating-induced partner preferences whereas males receiving a V_{1a} antagonist do not develop a partner preference, suggesting an involvement of VP in partner preference formation (Fig. 11a).

It appears that VP may be not only necessary but also sufficient for pair bond formation. Twenty-four hours of cohabitation with a female without mating does not induce a partner preference in male prairie voles. However, if males receive a central VP infusion, they exhibit a preference for a female partner even in the absence of mating (Fig. 11b; Winslow et al., 1993). VP infusions also induce aggression in male prairie voles. These effects appear specific, as OT at the same dose (0.5 ng/h) does not facilitate either partner preference formation or aggression in males. Curiously, central infusions of OT facilitated and infusions of the OT antagonist diminished partner preference formation in female prairie voles (Williams et al., 1994; Insel and Hulihan, 1995). In a recent study, a single injection of a high dose of VP or OT (100 ng) stimulated partner preference formation in both male and female prairie voles, but the temporal profiles of the peptide's effects need to be studied further (Cho et al., 1996).

Vasopressin regulation in male parental care

Mating with a female not only induces a pair bond but also enhances paternal responsiveness to a conspecific pup (Bamshad et al., 1994). This enhanced paternal responsiveness is associated with changes in central VP-ir staining and mRNA expression (Bamshad et al., 1994; Wang et al., 1994b). Male prairie voles that receive a septal VP injection exhibit a high level of paternal behavior and this VP effect is blocked by a pre-injection of a V_{1a} antagonist (Fig. 12a). In addition, males injected with the V_{1a} antagonist exhibited a lower level of paternal behavior than males injected with saline (Fig. 12b). Combined, these data suggest that VP acts on the V_{1a} receptors to influence male parental behavior in prairie voles (Wang et al., 1994a). Central VP pathways have been previously implicated in parental behavior in other rodents. Long–Evans rats, for example, display superior parental behavior in comparison with the VP-deficient mutant Brattleboro rats (Wideman and

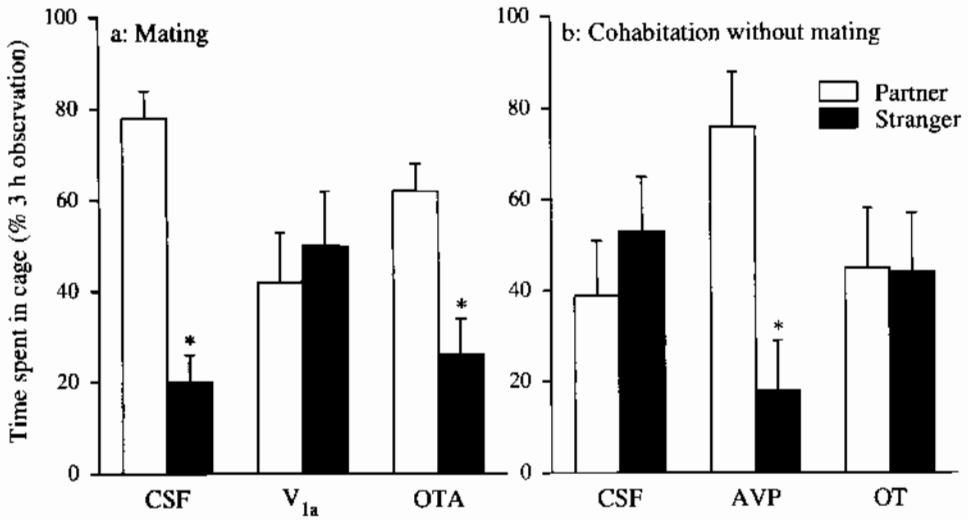


Fig. 11. Differences in preference of a partner versus strangers in male prairie voles that were either mated for 24 h (left) or cohabited with a female for 24 h without mating (right). Central injections of a V_{1a} antagonist (5 ng) diminished mating-induced partner preference whereas infusions of VP (0.5 ng/h) induced partner preference in the absence of mating. Scale bars, mean ± SEM; $*P < 0.05$ (adapted from Winslow et al., 1993).

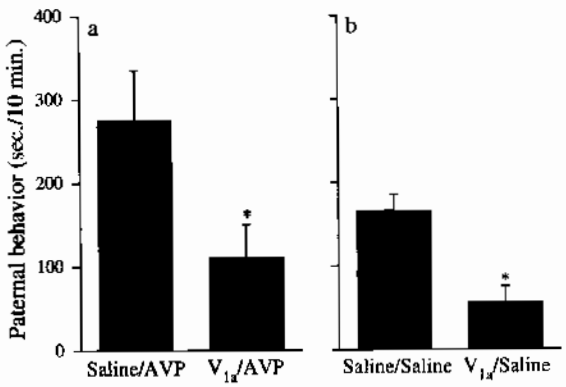


Fig. 12. Differences in paternal behavior between male prairie voles that received an injection of saline followed by an injection of VP (0.1 ng/100 nl saline) or a V_{1a} antagonist (1 ng/100 nl saline) followed by VP (a), or an injection of saline followed by saline or a V_{1a} antagonist followed by saline (b). Scale bars, mean ± SEM; $*P < 0.05$ (t test) (adapted from Wang et al., 1994a).

Murphy, 1990). Injections of VP into the lateral ventricle induces persistent parental behavior in female rats (Pedersen et al., 1982). It is not clear whether VP plays a role in initiation or maintenance of parental behavior in female prairie voles or non-monogamous voles of either sex.

Conclusions

Voles show remarkable differences in social behaviors associated with life strategy. Monogamous voles exhibit mating-induced pair bonding and biparental care, whereas promiscuous voles show neither pair bonding nor male parental care. These animals, thus, provide an ideal model system for the comparative studies of the neurobiological basis of social behavior. For example, by studying monogamous voles that exhibit remarkable behavioral changes during reproduction, one can explore neural mechanisms underlying changes of social behavior as well as the influence of complex behavior on the central nervous system. Although such approaches are still very recent, data from several studies have demonstrated the importance of VP in the regulation of social behaviors in voles.

Voles with different life strategy and behaviors show different distribution patterns of brain V_{1a} receptors, indicating differences in neural target sites for VP. This difference in receptor distribution could be due to species variation in the promoter sequences of the V_{1a} receptor gene. Monogamous and promiscuous voles also differ in the patterns of VP synthesis and release associated with reproduction. Mating in monogamous male voles induces an increase in extra-hypothalamic VP release and this released VP has been implicated in pair bonding and enhanced paternal care. Central injection/infusion of VP enhances pair bonding and male parental care whereas injections of the V_{1a} antagonist diminish these behaviors. Reproduction does not appear to influence central VP activity or social behaviors in promiscuous male voles. Finally, VP gene expression in the PVN and SON in male prairie voles does not change after mating but increases significantly with the onset of paternal care. Together these data suggest a dynamic mechanism by which VP regulates behavioral and physiological functions. On one hand, VP synthesis can be triggered by reproductive events and VP is released into specific central areas to regulate behaviors associated with reproduction. On the other hand, VP synthesis can also be increased in response to the altered physiological demands of social behavior. VP is, then, released peripherally or centrally to regulate physiological functions. Although studies on VP in voles are still at an early stage, the data, so far, have demonstrated voles as a useful model system for studying natural selection in social behavior, VP, and their interactions.

Acknowledgements

We thank Mr. Frank Kiernan for his professional assistance for photomicrographs. This article was written while Z.X. Wang was supported by NIMH 54554 and T.R. Insel was supported by NIMH 56539 and the Whitehall Foundation.

References

Albers, H.E., Hennessey, A.C. and Whitman, D.C. (1992) Vasopressin and the regulation of hamster social behavior. In: C.A. Pedersen, J.D. Caldwell, G.F. Jirikowski, and T.R. Insel (Eds.), *Oxytocin in Maternal, Sexual and*

Social Behaviors, Vol. 652. Academic Press, New York, pp. 227–242.

Bamshad, M., Novak, M.A. and De Vries, G.J. (1993) Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster*, and meadow voles, *Microtus pennsylvanicus*. *J. Neuroendocrinol.*, 5: 247–255.

Bamshad, M., Novak, M.A. and De Vries, G.J. (1994) Cohabitation alters vasopressin innervation and paternal behavior in prairie voles (*Microtus ochrogaster*). Physiol. *Behav.*, 56: 751–758.

Barberis, C. and Tribollet, E. (1996) Vasopressin and oxytocin receptors in the central nervous system. *Crit. Rev. Neurobiol.*, 10: 119–154.

Barberis, C., Balestre, M.-N., Jard, S., Tribollet, E., Arsenijevic, Y., Dreifuss, J.J., Bankowski, K., Manning, M., Chan, W.Y., Schlosser, S.S., Holsboer, F. and Elands, J. (1995) Characterization of a novel, linear radioiodinated vasopressin antagonist: An excellent radioligand for vasopressin V_{1a} receptors. *Neuroendocrinology*, 62: 135–146.

Baverstock, P. and Green, B. (1975) Water recycling in lactation. *Science*, 187: 657–658.

Bittman, E.L., Bartness, T.J., Goldman, B.D. and De Vries, G.J. (1991) Suprachiasmatic and paraventricular control of photoperiodism in Siberian hamster. *Am. J. Physiol.*, 260: R90–R101.

Bluthe, R.-M., Schoenen, J. and Dantzer, R. (1990) Androgen-dependent vasopressinergic neurons are involved in social recognition in rats. *Brian Res.*, 519: 150–157.

Boer, G.J., Buijs, R.M., Swaab, D.F. and De Vries, G.J. (1980) Vasopressin and the developing rat brain. *Peptides*, 1: 203–209.

Boer, G.J., van Rheenen-Verberg, C.M. and Uylings, H.B. (1982) Impaired brain development of the diabetes insipidus Brattleboro rat. *Dev. Brain Res.*, 3: 557–575.

Buijs, R.M., Swaab, D.F., Dogterom, J. and van Leeuwen, F.W. (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat: pathways to the limbic system, medulla oblongata and spinal cord. *Cell Tissue Res.*, 186: 423–433.

Caldwell, J.D., Greer, E.R., Johnson, M.F., Prange, A.J. and Pedersen, C.A. (1987) Oxytocin and vasopressin immunoreactivity in hypothalamic and extrahypathalamic sites in late pregnant and postpartum rats. *Neuroendocrinology*, 46: 39–47.

Carter, C.S. and Getz, L.L. (1993) Monagamy and the prairie vole. *Sci. Am.*, 268: 100–106.

Carter, C.S., Getz, L.L. and Cohen-Parsons, M. (1986) Relationships between social organization and behavioral endocrinology in a monogamous mammal. *Adv. Study Behav.*, 16: 109–144.

Cho, M.M., Dharmadhikari, A., Williams, J.R. and Carter, C.S. (1996) Comparisons of the behavioral effects of oxytocin and vasopressin in monogamous prairie voles. *Soc. Neurosci. Abstr.*, 22: 1151.

Compaan, J.C., Buijs, R.M., Pool, C.W., de Ruiter, A.J.H. and Koolhaas, J.M. (1993) Differential lateral septal vasopressin innervation in aggressive and non-aggressive male mice. *Brain Res. Bull.*, 30: 1–6.

Crenshaw, B.J., De Vries, G.J. and Yahr, P. (1992) Vasopressin innervation of sexually dimorphic structures of the gerbil forebrain under various hormonal conditions. *J. Comp. Neurol.*, 322: 1–10.

Crowley, R.S. and Amico, J.A. (1993) Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinology*, 133: 2711–2718.

Crowley, R.S., Insel, T.R., O'Keefe, J.A. and Amicos, J.A. (1993) Cytoplasmic oxytocin and vasopressin gene transcripts decline postpartum in the hypothalamus of the lactating rat. *Endocrinology*, 133: 2704–2710.

Crowley, R., Insel, T., O'Keefe, J., Kim, N. and Amico, J. (1995) Increased accumulation of oxytocin messenger ribonucleic acid in the hypothalamus of the female rat: induction by long term estradiol and progesterone administration and subsequent progesterone withdrawal. *Endocrinology*, 136: 224–231.

Dantzer, R., Koob, G.F., Bluthe, R.M. and Le Moal, M. (1988) Septal vasopressin modulates social memory in male rats. *Brain Res.*, 457: 143–147.

De Vries, G.J. (1990) Sex differences in neurotransmitter systems. *J. Endocrinol.*, 2: 1–13.

De Vries, G.J. and Buijs, R.M. (1983) The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. *Brain Res.*, 273: 307–317.

De Vries, G.J., Buijs, R.M., Van Leeuwen, F.W., Caffee, A.R. and Swaab, D.F. (1985) The vasopressinergic innervation of the brain in normal and castrated rats. *J. Comp. Neurol.*, 233: 236–254.

De Wied, D. (1977) Peptides and behavior. *Life Sci.*, 20: 195–204.

Dewsbury, D.A. (1987) The comparative psychology of monogamy. *Nebraska Symp. Motiv.*, 35: 1–50.

Emlen, S.T. and Oring, L.W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science*, 197: 215–223.

Ferris, C.F., Albers, H.E., Wesolowski, S.M., Goldman, B.D. and Leeman, S.E. (1984) Vasopressin injected into the hypothalamus triggers a stereotypic behavior in Golden hamsters. *Science*, 244: 521–523.

FitzGerald, R.W. and Madison, D.M. (1983) Social organization of a free-ranging population of pine voles,

Microtus pinetorum. *Behav. Ecol. Sociobiol.*, 13: 183–187.

Friedman, M.I. and Bruno, J.P. (1976) Exchange of water during lactation. *Science*, 197: 409–410.

Getz, L.L., Carter, S.C. and Gavish, L. (1981) The mating system of the prairie vole, *Microtus ochrogaster*: Field and laboratory evidence for pair-bonding. *Behav. Ecol. Sociobiol.*, 8: 189–194.

Gruder-Adams, S. and Getz, L. (1985) Comparisons of the mating system and parental behavior in *Microtus ochrogaster*, and *M. pennsylvanicus*. *J. Mammal.*, 66: 165–167.

Hermes, M.L.H.J., Buijs, R.M., Masson-Pevet, M. and Pevet, P. (1990) Seasonal changes in vasopressin in the brain of the garden dormouse (*Eliomys quercinusl*). *J. Comp. Neurol.*, 293: 340–346.

Higuchi, T., Tadokoro, Y., Honda, K. and Negoro, H. (1986) Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *J. Endocrinol.*, 110: 251–256.

Insel, T.R. and Hulihan, T.J. (1995) A gender-specific mechanism for pair bonding: Oxytocin and partner preference formation in monogamous voles. *Behav. Neurosci.*, 109: 782–789.

Insel, T.R. and Shapiro, L.E. (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci. USA*, 89: 5981–5985.

Insel, T.R., Wang, Z.X. and Ferris, C.F. (1994) Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *J. Neurosci.*, 14: 5381–5392.

Insel, T.R., Preston, S. and Winslow, J.T. (1995) Mating in the monogamous male: Behavioral consequences. *Physiol. Behav.*, 57: 615–627.

Irvin, R.W., Szot, P., Dorsa, D.M., Potegal, M. and Ferris, C.F. (1990) Vasopressin in the septal area of the golden hamster controls scent marking and grooming. *Physiol. Behav.*, 48: 693–699.

Jannett, F.J.J. (1980) Social dynamics of the montane vole, *Microtus montanus*, as a paradigm. *Biologist*, 62: 3–19.

Jannett, F.J.J. (1982) Nesting patterns of adult vole, *Microtus montanus*, in field population. *J. Mammal.*, 63: 495–498.

Jard, S. (1983) Vasopressin isoreceptors in mammals: Relation to cyclic AMP-dependent and cyclic AMP-dependent transduction mechanisms. In: A. Kleinzeller (Ed.), *Membrane Receptors*, Vol. 18, Academic Press, New York, pp. 255–285.

Johnson, A.E., Audiger, S., Rossi, F., Jard, S., Tribollet, E. and Barberis, C. (1993) Localization and characterization of vasopressin binding sites in the rat brain using an iodinated linear AVP antagonist. *Brain Res.*, 622: 9–16.

Kleiman, D. (1977) Monogamy in mammals. *Q. Rev. Biol.*, 52: 39–69.

Koolhaas, J.M., Van den Brink, T.H.C., Roosendaal, B. and Boorsma, F. (1990) Medial amygdala and aggressive behavior: Interaction between testosterone and vasopressin. *Aggressive Behav.*, 16: 223–229.

Le Moal, M., Dantzer, R., Michaud, B. and Koob, G.F. (1987) Centrally injected arginine vasopressin (AVP) facilitates social memory in rats. *Neurosci. Lett.*, 77: 353–359.

Madison, D.M. (1980) An integrated view of the social biology of *Microtus pennsylvanicus*. *Biologist*, 62: 20–33.

Mayes, C.R., Watts, A.G., McQueen, J.K., Fink, G. and Charlton, H.M. (1988) Gonadal steroids influence neurophysin II distribution in the forebrain of normal and mutant mice. *Neuroscience*, 25: 1013–1022.

McGuire, B. and Novak, M.A. (1984) A comparison of maternal behaviour in the meadow voles (*Microtus pennsylvanicus*), prairie voles (*M. ochrogaster*) and pine vole (*M. pinetorum*). *Anim. Behav.*, 32: 1132–1141.

McGuire, B. and Novak, M. (1986) Parental care and its relationship to social organizations in the montane vole (*Microtus montanus*). *J. Mammal.*, 67: 305–311.

Meisenberg, G. and Simmons, W.H. (1983) Centrally mediated effects of neurohypophysial hormones. *Neurosci. Biobehav. Rev.*, 7: 263–280.

Murasawa, S., Matsubara, H., Kijima, K., Maruyama, K., Mori, Y. and Inada, M. (1995) Structure of the rat V_{1a} vasopressin receptor gene and characterization of its promoter region and complete cDNA sequence of the 3′-end. *J. Biol. Chem.*, 270: 20042–20050.

Oliveras, D. and Novak, M. (1986) A comparison of paternal behaviour in the meadow vole *Microtus pennsylvanicus*, the pine vole *M. pinetorum*, and the prairie vole *M. ochrogaster*. *Anim. Behav.*, 34: 519–526.

Pedersen, C.A., Ascher, J.A., Prange, A.J.J. and Monroe, Y.L. (1982) Oxytocin induces maternal behavior in virgin female rats. *Science*, 216: 648–650.

Pittman, Q.J., Lawrence, D. and McLean, L. (1982) Central effects of arginine vasopressin on blood pressure in rats. *Endocrinology*, 110: 1058–1060.

Shapiro, L.E. and Dewsbury, D.A. (1990) Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of voles (*Microtus ochrogaster*, and *M. montanus*). *J. Comp. Psychol.*, 104: 268–274.

Shapiro, L.E. and Insel, T.R. (1990) Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. *Dev. Psychobiol.*, 23: 375–393.

Shapiro, L.E., Leonard, C.M., Sessions, C.E., Dewsbury, D.A. and Insel, T.R. (1991) Comparative neuroanatomy of the sexually dimorphic hypothalamus in monogamous and polygamous voles. *Brain Res.*, 541: 232–240.

Snijdewint, F.G.M., Van Leeuwen, F.W. and Boer, G.J. (1989) Ontogeny of vasopressin and oxytocin binding sites in the brain of Wistar and Brattleboro rats as demonstrated by light-microscopical autoradiography. *J. Chem. Neuroanat.*, 2: 3–17.

Thomas, A., Crowley, R.S. and Amicos, J.A. (1995 Effect of progesterone on hypothalamic oxytocin messenger ribonucleic acid levels in the lactating rat. *Endocrinology*, 136: 4188–4194.

Thomas, A., Kim, N. and Amico, J.A. (1996) Sequential exposure to estrogen and testosterone (T) and subsequent withdrawal of T increases the level of arginine vasopressin messenger ribonucleic acid in the hypothalamic paraventricular nucleus of the female rat. *J. Neuroendocrinol.*, 8: 793–800.

Tribollet, E., Goumaz, M., Raggenbass, M., Dubois-Dauphin, M. and Dreifuss, J.J. (1991) Early appearance and transient expression of vasopressin receptors in the brain of rat fetus and infant. An autoradiographical and electrophysiological study. *Dev. Brain Res.*, 58: 13–24.

Van Leeuwen, F.W., Caffe, A.R. and De Vries, G.J. (1985) Vasopressin cells in the bed nucleus of the stria terminalis of the rat: Sex differences and the influence of androgens. *Brain Res.*, 325: 391–394.

Van Leeuwen, F.W., van der Beek, E.M., van Heerikhuize, J.J., Wolters, P., van der Meulen, G. and Wan, Y.P. (1987) Quantitative light microscopic autoradiographic localization of binding sites labeled with [^{3}H] vasopressin antagonist d(CH_2)$_5$Tyr(Me)VP in the rat brain, pituitary and kidney. *Neurosci. Lett.*, 80: 121–126.

van Tol, H., Bolwerk, E., Liu, B. and Burbach, J. (1988) Oxytocin and vasopressin gene expression in the hypothalamo-neurohypophyseal system of the rat during the estrous cycle, pregnancy, and lactation. *Endocrinology*, 122: 945–951.

Wang, Z.X. (1995) Species differences in the vasopressin-immunoreactive pathways in the bed nucleus of the stria terminalis and medial amygdaloid nucleus in prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*). *Behav. Neurosci.*, 109: 305–311.

Wang, Z.X. and De Vries, G.J. (1993) Testosterone effects on paternal behavior and vasopressin immunoreactive projections in prairie voles (*Microtus ochrogaster*). *Brain Res.*, 631: 156–160.

Wang, Z.X. and Insel, T.R. (1996) Parental behavior in voles. *Adv. Study Behav.*, 25: 361–384.

Wang, Z.X., Ferris, C.F. and De Vries, G.J. (1994a) Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). *Proc. Natl. Acad. Sci. USA*, 91: 400–404.

Wang, Z.X., Smith, W., Major, D.E. and De Vries, G.J. (1994b) Sex and species differences in the effects of cohabitation on vasopressin messenger RNA expression in the bed nucleus of the stria terminalis in prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*). *Brain Res.*, 650: 212–218.

Wang, Z.X., Zhou, L., Hulihan, T.J. and Insel, T.R. (1996) Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: A quantitative comparative study. *J. Comp. Neurol.*, 366: 726–737.

Wang, Z.X., Young, L.J., Liu, Y. and Insel, T.R. (1997) Species differences in vasopressin receptor binding are evident early in development: Comparative anatomic studies in prairie and montane voles. *J. Comp. Neurol.*, 378: 535–546.

Wideman, C.H. and Murphy, H.M. (1990) Vasopressin, maternal behavior, and pup well-being. *Curr. Psychol. Res. Rev.*, 9: 285–295.

Wilkinson, M.F. and Kastling, N.W. (1987) The antipyretic effects of centrally administered vasopressin at different ambient temperatures. *Brain Res.*, 415: 275–280.

Williams, J.R., Insel, T.R., Harbaugh, C.R. and Carter, C.S. (1994) Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). *J. Neuroendocrinol.*, 6: 247–250.

Winslow, J.T., Hastings, N., Carter, C.S., Harbaugh, C.R. and Insel, T.R. (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, 365: 545–548.

Young, L.J., Winslow, J.T., Nilsen, R. and Insel, T.R. (1997) Species differences in V_{1a} receptor gene expression in monogamous and non-monogamous voles: Behavioral consequences. *Behav. Neurosci.*, 111: 599–605.

Zingg, H.H. and Lefebvre, D. (1988) Oxytocin and vasopressin gene expression during gestation and lactation. *Mol. Brain Res.*, 4: 1–6.

Zingg, H.H., Lefebvre, D. and Almanza, G. (1986) Regulation of vasopressin gene expression in rat hypothalamic neurons. *J. Biol. Chem.*, 261: 12956–12595.