REVIEWS

Vasopressin and Pair-Bond Formation: Genes to Brain to Behavior

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Microtine rodents provide an excellent model for the study of the neurobiology of social bonds. In this review, we discuss how the presence of a microsatellite sequence in the prairie vole vasopressin receptor gene may determine vasopressin receptor binding patterns in the brain and how these patterns may in turn affect social behavior.

Humans have evolved to rely on social bonds for survival. Consequently, the presence or absence of social support can directly impact one's health: social support reduces cardiovascular reactivity to acute psychological stress and is associated with better immune system function (49), whereas social isolation increases the risk for depression (52). A longitudinal epidemiological study also found that those who lacked social ties were 1.9–3.1 times as likely to die in a 9-yr period from a range of diseases, including ischemic heart disease, cerebrovascular and circulatory disease, cancer, and respiratory and gastrointestinal disease (3). On the opposite end of the spectrum, individuals who demonstrate severe deficits in the ability to form social bonds are significantly impaired in their ability to function normally in society. The autism spectrum disorders are examples of the latter. Hence, social behavior plays a crucial role in our survival as a species, and understanding its genetic and neural underpinnings may have important implications for human health.

Our laboratory investigates the neurobiological mechanisms underlying the formation of social bonds. To do this, one needs an appropriate animal model in which the formation of social bonds can be easily quantified. Microtine rodents, or voles, provide an excellent animal model for examining the neurobiological mechanisms underlying social bonding (60). Unlike 95% of mammalian species, prairie voles (*Microtus ochrogaster*) are socially monogamous, forming enduring and selective pair bonds with their mates. In the wild, pair-bonded prairie vole males reject unfamiliar, virgin females, and in <10% of cases do males abandon their female partner (23). If one partner in a breeding pair dies, fewer than 20% of the survivors eventually acquire a new mate (23). There is, however, individual variation in establishing pair bonds among prairie voles in nature, with approximately 45% of males and 24% of females adopting a wandering strategy and thus not settling down with a single partner (23). Prairie voles are also biparental, with both male and female parents taking equal partnership of raising their young. Interestingly, when population densities reach their maximum in the late autumn-winter season, 69% of prairie vole nests are communal, with reproductively inactive offspring remaining in the nest providing support for subsequent offspring (11, 23).

In the laboratory, pair-bond formation is assessed using a partner preference test (FIGURE 1) (11, 23, 53). After a 24-h period of cohabitation, male prairie voles are placed into a three-chambered testing arena in which the female partner is tethered in one chamber and a novel or "stranger" female in the other. The male is placed in a center, neutral cage and allowed to freely enter all cages. If a male spends more than twice as much time with his partner than the novel female, he is considered to have formed a selective partner preference. In our colony, over 90% of prairie vole males develop a selective partner preference after a 24-h cohabitation period with mating. Mating facilitates partner preference formation in males and females, although it is not essential for partner preference formation (11). In contrast to prairie voles, closely related meadow (*M. pennsylvanicus*) or montane (*M. montanus*) voles do not typically form partner preferences and actually spend most of their time in the neutral cage. This remarkable species difference in social bonding behavior provides an extraordinarily useful tool for investigating the neurobiological and genetic mechanisms underlying social bond formation (60).

The Molecules of Social Bonds: Vasopressin and Oxytocin

Social structures and behaviors vary widely between and within species. Such diversity implies *1*) rapid evolution of social behaviors and *2*) that the neural substrates underlying social behaviors are fairly plastic, demonstrating substantial individual variation within a species as well as variation across species. Furthermore, this degree of variation may arise from polymorphic genetic mechanisms that allow for rapid changes in the genetic expression of the neural substrates, and in turn social structure, from generation to generation. Our work in the neurobiology of pair-bond formation has generally supported these hypotheses. The bulk of the work on this subject has centered on the two neurohypophyseal hormones, oxytocin (OT) and arginine vasopressin (AVP).

OT and AVP are nonapeptides that differ in structure at only two amino acids (22). Both are synthesized

in the magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus, and these nuclei project to the neurohypophysis and release the hormones into the general circulation (9). The peripheral actions of OT include uterine contraction and milk ejection (10). The peripheral actions of AVP include blood pressure regulation (48) and antidiuretic properties (5). When released into the portal blood, AVP also acts as a secretagog of adrenocorticotropic hormone (47).

Although peripheral OT and AVP act as hormones, neuroanatomical mapping and tract tracing studies indicate that they also act as neurotransmitters or neuromodulators in the brain. OT and AVP synthesizing cells project to diverse sites throughout the brain (46), and their receptors are found throughout limbic and autonomic brain centers (2). Hence, the neuroanatomical distribution of OT and AVP fibers and their receptors suggests that they could play a role in regulating complex behavior. Indeed, a large body of work firmly establishes a role for OT and AVP in the regulation of social behaviors. OT modulates social interactions (57), aggression (56), and infant-mother attachments (39) and is essential for social recognition (15, 18, 19, 43). AVP regulates several aspects of social behavior, including social recognition (7, 14, 17, 45), social communication (26, 27), aggression (20, 55), scent marking (21), and paternal care (50).

Given the central role of OT and AVP in regulating social behavior, it was hypothesized that they may also regulate pair-bond formation. Both were found to do so [central infusions of OT facilitated pair-bond formation in the female prairie vole (12, 30, 54), whereas central infusion of AVP facilitated pair-bond formation in male prairie voles], even in the absence of mating (12, 55). In addition, infusion of OT and AVP antagonists prevent pair-bond formation. Interestingly, similar infusions of AVP into non-monogamous male montane voles do not facilitate pair-bond formation (59), despite the fact that the peptide distribution is similar between species (51). These data suggest that differences in the actions of OT and AVP at their receptors between the two species may underlie the differences in pair bonding. In fact, elegant brain receptor autoradiography studies revealed that the brain receptor distribution patterns for the OT (31) and vasopressin 1a (V1aR) receptors (32, 58) are remarkably different between species (FIGURE 2). Hence, the differential effects of intracerebroventricular infusions of the OT and AVP are more than likely due to the different brain locations of the two receptors across different vole species.

These data indicate that centrally acting AVP and OT regulate pair-bond formation and that receptor distribution patterns may account for the different patterns of social behavior between closely related species. In the rest of this review, we will discuss the underlying genetics of the brain distribution patterns
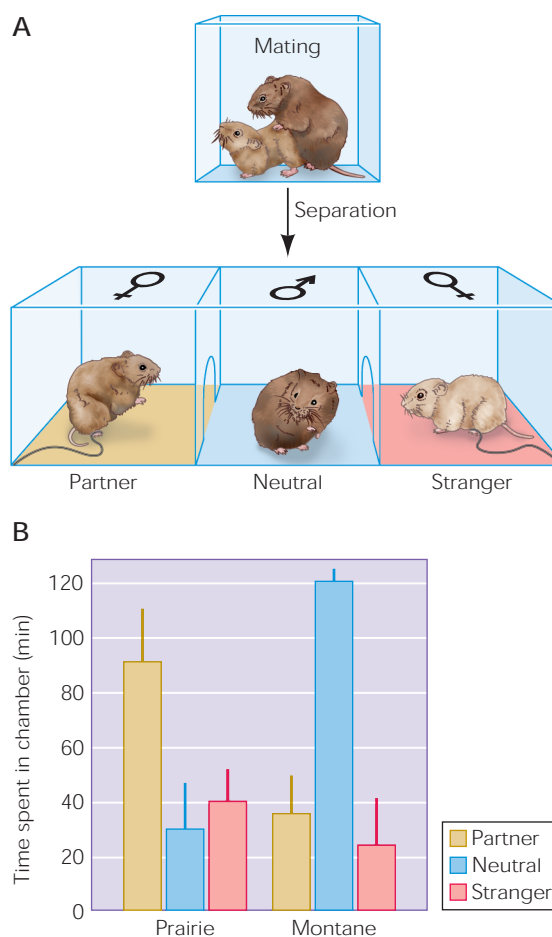

FIGURE 1. **Partner preference test**
A: a male and female vole are mated for 24 h. They are then separated, and the male is placed in a three-chambered apparatus and given a choice between the partner female, a stranger female, or a neutral area. Females are tethered to their respective chambers. *B*: whereas prairie voles spend significantly greater time with their partner, montane voles spend most of their time in the neutral cage area.

of the V1aR and how this brain distribution is linked to pair bonding in male prairie voles.

Genetics of V1aR Brain Distribution Patterns

There are no species differences in binding kinetics or second messenger coupling of the V1aR between montane and prairie voles (32), and the coding region of the V1aR gene (*avpr1a*) is 99% identical (59). Therefore, the observed binding differences must be linked to a genetic difference outside of the coding region. In support of this, transgenic mice created using 2.2 kb of the 5′ regulatory region of the prairie vole V1aR demonstrated patterns of V1a expression very similar to that of prairie voles. Interestingly, intracerebroventricular administration of AVP increased affiliative behavior in mice homozygous for the prairie vole regulatory region.

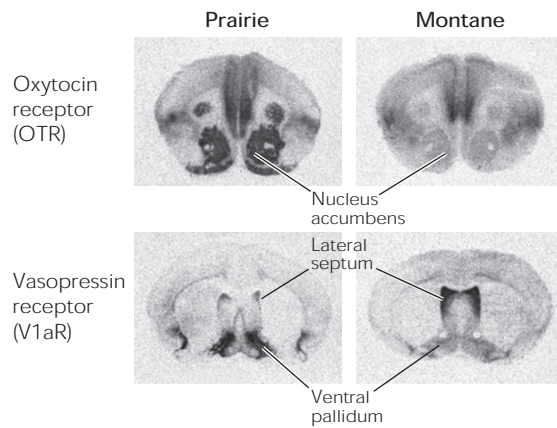Subsequent sequencing studies of the *avpr1a* loci in

FIGURE 2. OTR and V1aR receptor distribution patterns differ between prairie and montane voles
Prairie voles have a higher OTR density in the accumbens. Prairie and montane voles have different V1aR densities in the lateral septum (LS) and ventral pallidum (VP).

montane and prairie voles revealed two important differences between the species. First, the prairie vole genome has two copies of the *avpr1a* gene, whereas the montane vole genome has only one. One of the prairie vole loci has a truncated long interspersed nuclear element (LINE) in the 5′ regulatory region (59). LINE elements may cause surrounding DNA sequences to be duplicated and translocated to a different part of the genome (38). The altered chromosomal environment could then allow for different transcriptional regulation of gene. In the other loci, a single base mutation results in a premature stop codon and thus a truncated form of the receptor. It is not clear which of the loci may be the translocated or duplicated one. Montane voles have only one V1aR loci and no LINE elements.

The second important species difference in the vole *avpr1a* loci is the presence of repetitive di- and tetranucleotide sequences between 720 and 1,150 bp upstream of the transcriptional start site (FIGURE 3A). These sequences constitute a "microsatellite element," which is largely absent in the montane vole.
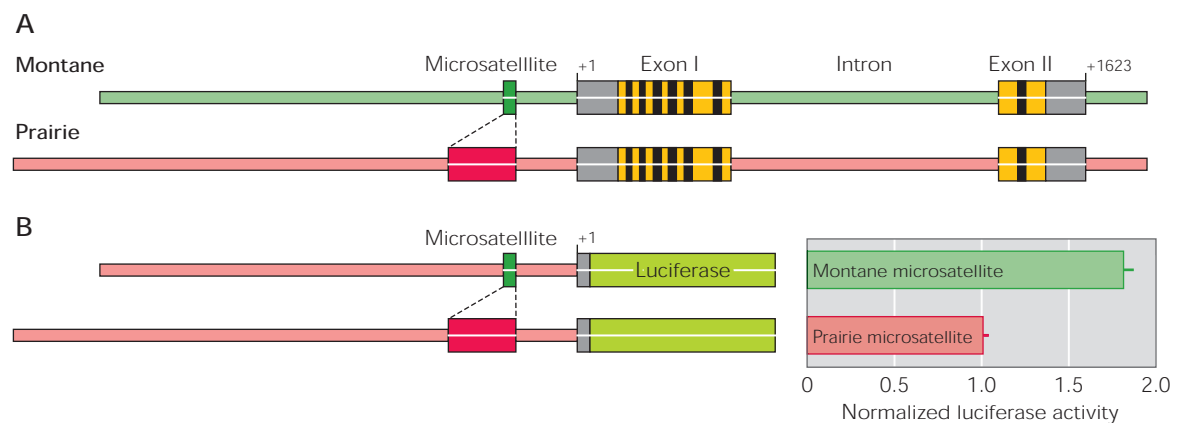
Interestingly, the monogamous pine vole (*Microtus pinetorum*) has a similar microsatellite, whereas the promiscuous meadow vole does not. Therefore, aside from a LINE-induced translocation/duplication event, interspecies variation in V1aR may be due to the presence of the microsatellite sequence. Hence, there are two potential molecular events that could have led to the divergent expression patterns in the vole species: *1*) the LINE-induced translocation/duplication event and *2*) the species differences in microsatellite length and composition.

The hypothesis that the *avpr1a* microsatellite region could alter the expression pattern of the V1aR was first examined in cell culture. Using different rat cell lines with endogenous expression of the V1aR to model the phenotypic diversity of cells in the brain, Hammock and Young (28) demonstrated that the presence of the montane or prairie vole microsatellite could potentially alter *avpr1a* expression in a cell-type-specific manner. Specifically, different cell lines transfected with luciferase-reporter plasmids under the regulatory control of the *avpr1a* regulatory sequence from the prairie vole showed differential luciferase expression depending on whether the prairie or montane microsatellite was present. In some cell lines, like the A7r5 (derived from the smooth muscle of the thoracic aorta) and PC12 cells (from adrenal gland pheochromocytoma), the presence of the montane microsatellite increased luciferase activity relative to the prairie vole microsatellite (FIGURE 3B), whereas in other cell lines, like the H4IIE (liver hepatoma) and A10 (from smooth muscle of the thoracic aorta), luciferase activity did not change. These data suggest that the microsatellite may affect transcriptional regulation of the V1aR depending on the particular cellular environment in which it is located.

How in particular might the microsatellite confer cell-type-specific regulation of microsatellite? Hammock and Young (28) speculate that the repetitive nature of the microsatellite may result in structural changes in the surrounding DNA that could alter tran-

FIGURE 3. The structure of the *avpr1* gene of prairie and montane voles
A: the genes for montane and prairie voles share a high degree of homology except for an expanded microsatellite sequence in the 5′ flanking region of the prairie vole gene. The green and dark red boxes indicate the relative length and position of the microsatellite.

scription. Another possibility is that the microsatellite may be positioned between interacting transcription factors so that the length of microsatellite physically prevents interactions that modulate expression. Finally, the microsatellite itself could contain a regulatory element binding site that directly affects expression. In future studies, we plan to study how structural differences in *avpr1a* microsatellite might change V1aR distribution patterns. These studies should provide valuable insights into the role of microsatellite elements in the evolution of gene expression as well as to the mechanisms by which mutations in these elements affect expression.

Microsatellite Polymorphisms and Individual Variation in V1aR Distribution

Field-caught prairie voles demonstrate an extraordinary amount of individual variation in the density of V1aR binding in specific brain regions. Phelps and Young (41) examined V1aR binding across the olfactory bulb, extended amygdala, thalamus, cingulate cortex, ventral pallidum, and their subnuclei, and found approximately a twofold difference in V1aR binding between the lower and upper quartiles of 32 sampled animals. Some regions, like the medial geniculate nucleus of the thalamus, showed up to a 10-fold difference. Many of the observed differences were as large as those we see between vole species. Whether the presence of the microsatellite could account for such individual variation in V1aR patterning within the prairie vole species has been the focus of subsequent studies from our laboratory. Specifically, since the length of the microsatellite itself can alter the degree of luciferase reporter in cell culture [microsatellites differing by only 19 bp showed differential luciferase reporter activity (29)], it was hypothesized that individual variation in the length of the microsatellite may account for the individual variation in binding differences. In turn, these differences in binding could be linked to individual variation in social behaviors.

To test these hypotheses, Hammock and Young (29) created two breeding lines of prairie voles, one with the long *avpr1a* microsatellite allele and another with the short allele (FIGURE 4A). The genotype differences were associated with robust differences in V1aR binding but varied with the particular region of interest (FIGURE 4B). For instance, long-alleled animals showed higher levels of receptor binding in the olfactory bulb and lateral septum but lower levels in the amygdala, hippocampus, and posterior hippocampus compared with short-alleled animals. Furthermore, there were some regions like the ventral pallidum that showed no genotype differences in binding. Hence, these data support the hypothesis generated by the aforementioned cell-culture studies, namely that the presence of the microsatellite regulates V1aR expression in a cell-type-specific manner.

With respect to behavior, long-alleled breeding males exhibited greater pup-licking and grooming, indicative of greater paternal behavior compared with short-alleled males. F1 generation long-alleled male offspring showed a shorter latency to approach a social odor, greater prosocial behavior, and stronger partner preferences than short-alleled animals (FIG-
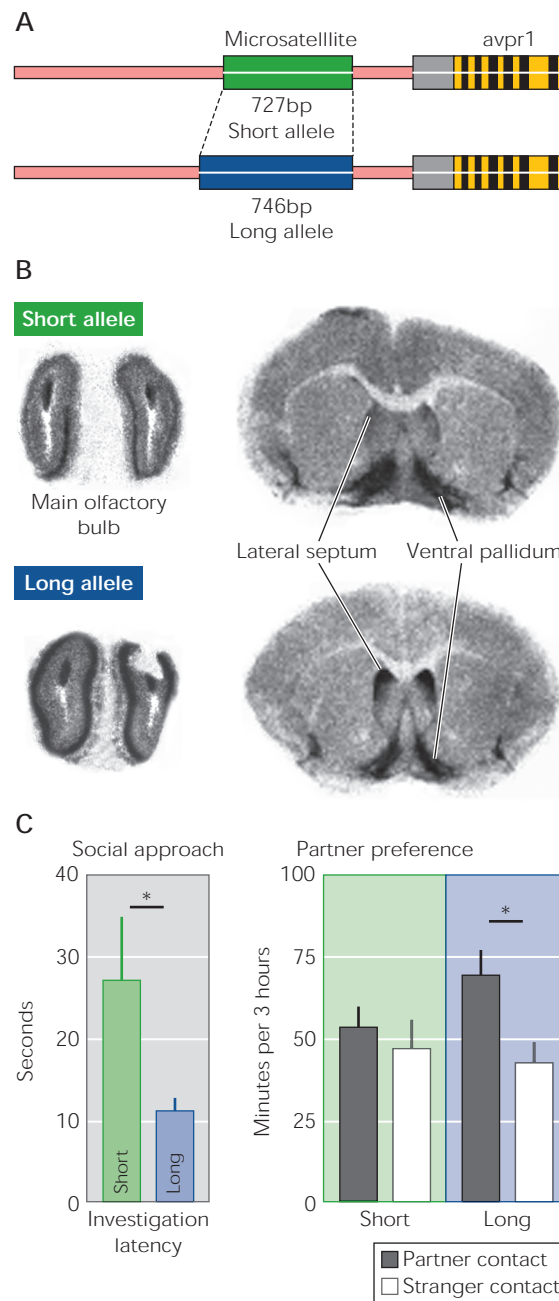
FIGURE 4. **Short vs. long allele comparison**
A: two different common alleles of the prairie vole microsatellite differ in length by 19 base pairs. *B*: prairie voles selectively bred for the short or long alleles have different V1aR binding densities in the main olfactory bulb, lateral septum, and ventral pallidum. *C*: short- and long-alleled male prairie voles demonstrate different social behavior. The latency to approach and investigate a novel juvenile male was shorter among long-alleled males. Long-alleled voles demonstrated partner preferences, whereas short-alleled males did not.

URE 4C). Interestingly, no behavioral differences were found between female long- and short-alleled prairie voles, which is consistent with the fact that AVP regulates primarily male species typical behavior and not female behavior.

In summary, these data point to a causal chain of events that lead to large variations in social behavior across species and individuals: a genetic polymorphism in the 5′ regulatory region of the *avpr1* gene alters V1aR expression throughout the brain in a cell-type (and thus region-specific) manner. In turn, the functional consequences of AVP transmission throughout the brain can be entirely altered. The fact that individuals within a species show variation in microsatellite length indicates the instability of the microsatellite sequence and may account for the large variation in V1aR receptor binding. Such plasticity presumably allows for rapid evolution of the V1aR system to accommodate rapidly changing ecological needs. In the next section, we discuss where in the brain V1aR receptors may be linked to pair-bond formation.

Vasopressin, Ventral Pallidum, and Pair Bonding

In male prairie voles, neuropeptide receptor mapping (61), pharmacological studies (36), and genetic manipulations (35, 42, 59) have demonstrated that AVP acting through the V1aR receptor plays a crucial role in pair-bond formation. Despite the numerous differences in brain V1aR receptor expression, one region where V1aR binding consistently distinguishes monogamous and non-monogamous species is the ventral pallidum. This is interesting because the ventral pallidum, a major output region of the accumbens, is known to regulate reward-related behavior via dopamine-related mechanisms. Lesioning the ventral pallidum disrupts rewarding properties of food (13). Conditioned place preference can be induced by directly infusing psychostimulants into the ventral pallidum (25), whereas depletion of dopamine in the ventral pallidum blocks this effect (24). Interestingly, positron emission tomography imaging reveals that sexual and competitive arousal in normal men is correlated with increased regional cerebral blood flow in the ventral pallidum (44). By virtue of its receipt of projections from the rostral pole and shell of the accumbens and its efferents to the ventral tegmental area and mediodorsal thalamus (62), the ventral pallidum is thought to be in a crucial position to integrate "reinforcement and reward" signals with cortical systems of cognitive representation (4).

Monogamous species such as the prairie vole, California mouse (*Peromyscus californicus*), and common marmoset (*Callithrix jacchus*) express higher levels of V1aR in the ventral pallidum relative to non-related monogamous species, suggesting that AVP,

acting through the V1aR in the ventral pallidum, may be an important mediator of pair-bond formation. In support of this hypothesis, the ventral pallidum contains vasopressin fiber immunoreactivity (33), and viral vector-mediated increases in V1aR receptors in the prairie vole ventral pallidum (42) facilitate pair-bond formation, whereas site-specific injections of a V1aR antagonist (36) reduce it. Remarkably, when the prarie vole V1aR receptors (the coding region) are overexpressed in the ventral pallidum in the non-monogamous meadow vole, they display partner preferences (35), an effect that can be reversed with the dopamine D2 receptor antagonist eticlopride. These data, along with the fact that pair bonding is dependent on dopamine signaling in the accumbens (1), suggest to us that pair-bond formation is fundamentally a reward-related process, involving vasopressin-modulated dopaminergic signaling in the ventral pallidum.

However, V1aR activation in the ventral pallidum is unlikely to be the only mechanism involved in pair-bond formation. For one, although the strength and propensity to form a pair bond is predicted by *avpr1a* microsatellite length (29), *avpr1a* microsatellite length is not correlated with ventral pallidum V1aR binding. Hence, other regions whose V1aR binding varies with microsatellite length are likely to play a role in pair-bond formation. The lateral septum could be a candidate region. In fact, it has been found that AVP facilitates, whereas V1aR antagonists block, partner preference formation when infused into the lateral septum (37). Furthermore, it is possible that other factors could be involved. For example, an OT receptor antagonist in the lateral septum can also block mating- or AVP-induced partner preference formation, indicating that pair-bond formation in males is not solely dependent on V1aR receptor (37). Alternatively, since replacement of V1aR receptors into the lateral septum of V1aR knockout mice rescues social recognition deficits (6), AVP could be regulating the social discrimination aspects of pair bonding, whereas the rewarding aspects are mediated by the ventral pallidum. Detailed studies have yet to be performed to dissociate these different aspects of pair-bond formation and delineate which regions preside over them.

Pair-Bond Formation vs. Maintenance of a Pair Bond

Although much variation is found in V1aR binding across several brain regions in wild-caught prairie voles, the ventral pallidum shows among the lowest variation. If ventral pallidal V1aR is necessary and sufficient for pair bonding, then there should also be little variability in pair-bond formation in the wild. Contrary to this, during certain seasons, ~45% of male prairie voles may take up a wandering strategy, mating with multiple females. Again, these data suggest that ventral pallidal V1aR is not the only circuitry mediat-

ing pair-bond formation. Alternatively, it could be argued that, although this 45% are capable of forming a pair bond, they may differ in their capacity to sustain it; i.e., perhaps the pair bond is not enduring. If so, this raises the possibility that formation versus maintenance of the pair bond may be under the control of separable brain mechanisms.

In fact, it has long been proposed that social bonds may be governed by a "bi-dimensional" system, one that elicits aversive feelings when social bonds are disrupted on the one hand and gratifying feelings in the presence of those bonds on the other (40). The former may provide a crucial, adaptive mechanism for maintaining social bonds much in the same way that pain systems protect one from injury or death. Given that remaining with a single partner is thought to be crucial to prairie vole fitness because of the relatively sparse and dispersed food sources of their habitat, a similar distress/mechanism may be in place to maintain the pair bond. Hence, although a VP-/V1aR-mediated reward mechanism may be crucial to the formation of the pair bond, the maintenance of it may rely on an aversive social distress mechanism.

We recently examined male prairie voles after separation from their female partner following 5 days of cohabitation. Specifically, we assessed active/passive coping strategies in the forced swim and tail-suspension tests, two of the hallmark tests often used in animal models of depression. Remarkably, male prairie voles separated from their partner, but not from a sibling, demonstrated significantly increased passive behavior on both tests, indicating that the loss of a partner is quite aversive to male prairie voles (8). In many ways, the findings appear to parallel the depression or grieving experienced by humans during bereavement.

These data indicate that there does appear to be a separate mechanism, possibly involving neural systems mediating anxiety and depression, that serves to maintain the pair bond. A candidate system underlying the maintenance of the pair bond could be the corticotropin-releasing factor (CRF) system, since it has been shown to be linked to anxiety, depression, and aversive responses to drug withdrawal. In support of this hypothesis, central infusions of CRF in male prairie voles before cohabitation with a female results in the facilitation of a partner preference (16), and in a recent mapping study of the two known CRF receptors, CRF1 and CRF2, remarkable species differences were found between prairie and meadow voles in receptor-binding distributions throughout the brain (34). Hence, the CRF system is likely to play a role in pair bonding and perhaps specifically in the maintenance of the pair bond.

Summary and Conclusions

The vasopressin receptor distribution patterns in the brain in part determine the social structure of species like voles. The plasticity of these patterns appears to arise from the instability of a microsatellite sequence in the 5′ regulatory region of the *avpr1a* gene. Hence, the rapid evolution of social behavior can be linked to genetic polymorphisms in the genes for the receptors of neuropeptides like vasopressin. Could a similar mechanism occur in humans? Interestingly, four polymorphic microsatellites surround the human *avpr1a* gene, and sequences in and around these regions are different in other non-human primates, like the chimpanzee (29). Whether these sequences in humans could account for our variation in social behavior and whether they could be linked to disorders like autism are important questions yet to be explored. ■

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