

Neurochemical regulation of pair bonding in male prairie voles

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Abstract

Pair bonding represents social attachment between mates and is common among monogamous animals. The prairie vole (*Microtus ochrogaster*) is a monogamous rodent in which mating facilitates pair bond formation. In this review, we first discuss how prairie voles have been used as an excellent model for neurobiological studies of pair bonding. We then primarily focus on male prairie voles to summarize recent findings from neuroanatomical, neurochemical, cellular, molecular, and behavioral studies implicating vasopressin (AVP), oxytocin (OT), and dopamine (DA) in the regulation of pair bonding. Possible interactions among these neurochemicals in the regulation of pair bonding, the brain areas important for pair bond formation, and potential sexually dimorphic mechanisms underlying pair bonding are also discussed. As analogous social bonds are formed by humans, investigation of the neurochemical regulation of pair bond formation in prairie voles may be beneficial for our understanding of the mechanisms associated with normal and abnormal social behaviors in humans.

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Keywords: Vasopressin; Oxytocin; Dopamine; Pair bonding; Monogamy

1. Introduction

While several types of social behaviors such as aggression, sex, and social separation have been the focus for many studies in behavioral neuroscience, the neurobiology of pair bonding, a special type of social attachment between mates, has been largely unexplored. The lack of previous research in this area may be partly explained by the complexity of pair bonding, which involves, but is not limited to, sensory processing, memory, motivation, and more subtle aspects of behavior that may be difficult to measure. In addition, neurobiological studies of pair bonding require an animal model demonstrating a reliable behavioral index of pair bond formation. Unfortunately, traditionally studied laboratory rodents, such as rats and mice, generally do not display social attachment between mates and thus cannot be used to study pair bonding. Over the past several years, studies focusing on the development of pair bonds in a microtine rodent, the prairie vole (*Microtus ochrogaster*), have investigated the hormonal, neuroanatomical, cellular and molecular regulation of pair

bonding. In these studies, a pair bond is defined as a stable relationship between a breeding pair of animals that share common territory and parental duties. As analogous social bonds are formed by humans, and the inability to form such bonds is a key diagnostic component in certain psychological disorders [76], these results are important, not only for comprehensive understanding of the neural regulation of pair bonding, but also for our understanding of the mechanisms underlying social disorders in humans. In this review, we will first introduce the prairie vole model, and then primarily focus on studies using male prairie voles to review recent findings on the neurochemical regulation of pair bonding. In this regard, we will focus on the neuropeptides vasopressin (AVP) and oxytocin (OT) and the monoamine neurotransmitter dopamine (DA). However, it is important to note that several other neural [29], hormonal [23], chemical [9], and environmental factors [24] are also involved in the regulation of pair bond formation.

2. Prairie vole model for pair bonding

The prairie vole belongs to the genus *Microtus* within the family *Muridae* (subfamily *Arvicolinae*) [1], and lives

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primarily in the grasslands of the central United States. Both field and laboratory studies have indicated that prairie voles are monogamous. In the field, male and female prairie voles form long-term bonds and share a nest throughout the breeding season [30,31,39,40]. Such a breeding pair typically remains together until one dies [38]. In the laboratory, both sexes mate preferentially with one partner [31]. Prior to mating, males and females exhibit non-selective affiliative behavior. However, after multiple mating bouts within a 24-h period, both the male and female show behavioral changes indicative of pair bonding. For instance, the mated pair shares a nest, remains together during gestation, and displays extensive parental care throughout lactation [59,61]. Pair-bonded males also show increased aggressive behavior towards conspecific strangers, but not the familiar partner [46,80,91], and this behavior serves to guard mate and territory [15]. It should be pointed out that although extended cohabitation in the absence of mating induces pair bond formation in female prairie voles [89], 18–24 h of mating seem to be necessary for pair bond formation in males [46,91].

An important behavioral characteristic associated with pair bonding is that after mating, prairie voles display a robust preference for the familiar mate versus a conspecific stranger. This preferential association with the familiar mate can be quantified using a partner preference test, first developed in Dr. Sue Carter's laboratory [88] and subsequently adopted by several other labs, including our own. Although specific aspects of the paradigm may differ across laboratories, the general concept is the same. A three-chamber testing apparatus consists of a central cage with tubes connecting it with two identical cages, one containing the partner and the other a conspecific stranger. These two stimulus animals are tethered in their own cages and do not interact with each other, whereas the subject is free to move throughout the testing apparatus during a 3-h partner preference test. In our lab, a customized computer program using a series of light beams across the connecting tubes monitors subject movement among the cages and time spent in each cage. Pair bonding is inferred when subjects spend significantly more time in contact with their partners than with strangers. For prairie voles, it has been demonstrated that 24 h of mating reliably results in partner preference formation, whereas 6 h of cohabitation in the absence of mating does not induce this behavior [46]. This paradigm has been successfully used in conjunction with pharmacological manipulations to study the neurochemistry of pair bonding [84,98]. For example, if drug treatment prior to pairing prevents mating-induced partner preference formation, it indicates reliance of pair bond formation on the impacted neurochemical system. On the other hand, the ability of a drug to induce partner preference in animals that are housed together for 6 h in the absence of mating suggests that stimulation of the relevant receptors is sufficient for pair bond induction.

Finally, while prairie voles are considered to be monogamous, other species of voles, such as montane

(*Microtus montanus*) and meadow voles (*Microtus pennsylvanicus*) that are taxonomically close to prairie voles, have non-monogamous life strategies and are asocial. In these latter species, individuals show low levels of social affiliation, do not mate preferentially with one partner and display no pair bonding after mating, and only females provide parental care [31,46,50,58,61]. These additional vole species, together with prairie voles, provide excellent opportunities for comparative studies of the neurobiology of pair bonding. It has also been demonstrated that prairie voles from different geographic locations differ in some aspects of social behavior. For example, although prairie voles captured in Kansas display mating-induced partner preference formation, they show some differences in sexual dimorphism, parental behavior, and behavioral responsiveness to vasopressin, in comparison to their counterparts from Illinois [21,67,68]. In the present review, we will focus on prairie voles originally captured in Illinois, as they have been used in the majority of studies examining neurochemical regulation of pair bonding.

3. Neuropeptide regulation of pair bonding

Early studies examining the neurobiological basis of pair bonding were primarily focused on the neuropeptides arginine vasopressin (AVP) and oxytocin (OT). These neuropeptides were chosen because they had been implicated in several social behaviors, including sexual behavior [4,13], parental care [44,52,62,63], aggression [17,36,37], and territory marking [49]. In addition, they were found to be involved in learning/memory and individual recognition—processes important for pair bond formation and expression [14]. For example, central AVP, particularly AVP in the lateral septum (LS), has been found to be important for individual recognition in rats [10,33,54,66]. Similarly, central OT has been implicated in social memory in a variety of different paradigms [33,35,92].

3.1. AVP and OT cells and projections

Studies of central AVP and OT systems in voles began with experiments examining central AVP- and OT-producing cells and their projections, and correlating differences in these systems with different life strategies and social behaviors among vole species. In addition to species comparisons, sex differences in these neural systems have also been closely examined. These data have been reviewed elsewhere in detail [28,84]; here, we briefly summarize the findings. Despite dramatic differences in social behavior, prairie voles do not differ significantly from non-monogamous voles in the distribution pattern of AVP or OT cells and projections. However, there are major differences in the AVP systems between males and females [5,77,82,87]. In addition to hypothalamic nuclei, AVP cells are found in the

bed nucleus of the stria terminalis (BST) and the medial nucleus of the amygdala (MeA), while AVP immunoreactive (ir) projections are found in several brain areas including the LS and lateral habenular nucleus (LH). In comparison to female voles, males have more AVP-producing cells in BST and MeA and a higher density of AVP-ir projections in LS and LH. In addition, this AVP pathway is gonadal steroid dependent: castration reduces the number of AVP cells in BST and MeA and the density of AVP-ir fibers in LS and LH, whereas testosterone replacement reverses this effect [78]. This sexually dimorphic and steroid-dependent AVP pathway resembles those reported in other species of rodents [18,27,75]. OT-ir cells are also found in the hypothalamus, the BST, and the medial preoptic area, and their distribution is similar between males and females [87].

A finding of major significance for pair bonding is that social experience alters the central AVP system, particularly the AVP pathway from BST to LS. Three days of cohabitation with a female (during which mating typically occurs) induces an increase in AVP mRNA in BST and a decrease in AVP-ir staining in LS in male prairie voles [6,82]. Similar effects are not found in female prairie voles, and 3 days of experience with an opposite sex individual does not alter AVP mRNA in BST in a non-monogamous vole. In rats, AVP cells in BST project to LS [26]; however, effects of sexual experience on AVP expression in rats have not been tested. If AVP cells in BST also project to LS in voles, this gender- and species-specific increase in AVP mRNA in BST, with a decrease in AVP-ir staining in LS, in male prairie voles may indicate mating-induced AVP release from LS. The released AVP, in turn, may be involved in the mating-induced behavioral changes in male prairie voles [84].

3.2. AVP and OT receptors

While the above data show that distribution pattern and amount of AVP and OT are similar across vole species, there are dramatic species differences in the distribution patterns and region quantities of AVP and OT receptors (Fig. 1). For example, for AVP V1a receptor binding and mRNA labeling [48,85,99], prairie voles have more V1a receptors in BST, ventral pallidum, and thalamus, whereas montane voles have more in LS and ventromedial hypothalamus (VMH). This species difference is true for both males and females. Prairie voles also have more OT receptors in the nucleus accumbens (NAcc), prelimbic cortex, BST, and lateral amygdala, whereas montane voles have more in LS, VMH, and cortical amygdala [47,95]. Some of these differences in the V1a and OT receptors are present at birth, while others change over the course of postnatal development [83,85]. These data suggest that monogamous and non-monogamous voles differ in brain responsiveness to centrally released AVP and OT. It is important to note that these species differences in AVP and OT receptors are specific, as no species differences are found in benzodiazepine or μ -opioid receptors [47]. Overall, no noticeable sex differences are found for the baseline levels of the central AVP and OT receptors. OT receptors fluctuate with the various stages of reproduction; they increase in the lateral amygdala of female montane voles and VMH of both female prairie and montane voles within 24 h of parturition [47,81]. However, AVP receptor expression appears to be stable during reproduction in both males and females of monogamous and non-monogamous voles [81].

Molecular studies have also shown that the AVP and OT receptor genes of monogamous and non-monogamous voles have almost identical coding regions, but their promoter

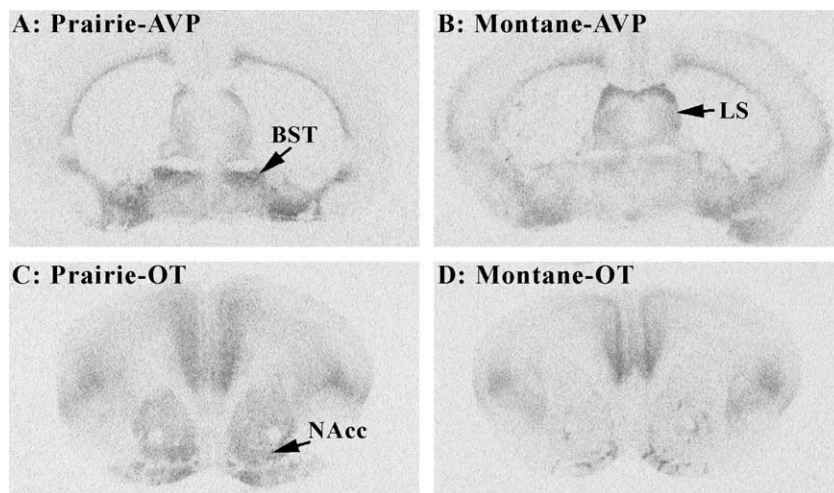


Fig. 1. Photomicrographs from autoradiograms displaying the binding for vasopressin V1a (AVP) and oxytocin (OT) receptors in brain sections from prairie and montane voles. The density of the V1a receptor binding is higher in the bed nucleus of the stria terminalis (BST) and lower in the lateral septum (LS) in prairie voles (A), in comparison to montane voles (B). Furthermore, prairie voles (C) have a higher density of oxytocin receptor binding in the nucleus accumbens (NAcc) than montane voles (D).

regions differ [95,99]. These data suggest that voles with different life strategies and social behaviors express the same AVP and OT receptors, but differences in the gene promoters may be sufficient to drive the species-specific receptor distribution in the brain. The functional significance of the neuropeptide receptor distribution has been demonstrated in two elegant studies. Mice that are transgenic for the prairie vole V1a receptor gene show the distribution pattern of the V1a receptors similar to that of prairie voles, and these mice exhibit increased affiliative behavior in response to central AVP administration [97]. Additionally, using an adeno-associated viral vector delivery of the V1a receptor gene, AVP receptor expression was increased specifically in the ventral pallidum in male prairie voles, and these males exhibited increased levels of affiliative behavior and enhanced partner preference formation compared to the control males [65]. These data demonstrate that the pattern and regional quantity of the neuropeptide receptors have important functional significance in the regulation of social behavior.

3.3. *Neuropharmacological testing*

An early study examining neuropeptide regulation of pair bonding was performed in male prairie voles [91]. Males that mated with their partner for 24 h showed partner preferences; however, this behavior was prevented if subjects received intracerebroventricular (icv) injections of the V1a receptor antagonist. Similar administration of an OT receptor antagonist did not prevent partner preferences. Further, males that received icv infusions of AVP, but not OT, displayed partner preferences after 6 h of cohabitation with a female in the absence of mating. In the same study, AVP, but not OT, was also found to regulate selective aggression in male prairie voles. It is important to note that such AVP manipulation neither influenced mating or social interactions during the initial cohabitation/mating period nor altered locomotor activity during the partner preference test, indicating the specificity of AVP effects on pair bond formation. Additional studies suggested that OT, but not AVP, regulates pair bonding in female prairie voles [45,90]. Therefore, these behavioral data, along with differences in AVP neuroanatomy between males and females, suggest a sexually dimorphic mechanism for the neuropeptide regulation of pair bonding—AVP regulates pair bonding in males, whereas OT regulates the same behavior in females.

Recent studies, however, have shown that the AVP/OT regulation of pair bonding in prairie voles may not be so straightforward. In a study in which a wider range of doses of AVP and OT was used, icv administration of either AVP or OT was found to induce partner preference formation in both male and female prairie voles following only 1-h cohabitation, indicating that both neuropeptides can be involved in pair bond formation in both sexes of prairie voles [16]. This notion is further supported by a recent study from our lab [56]. Site-specific administration of either the

V1a receptor antagonist or OT receptor antagonist into LS blocked mating-induced partner preferences in male prairie voles. In addition, OT receptor antagonist in LS also blocked partner preferences induced by AVP, indicating possible interactions of AVP and OT in pair bond formation. Discrepancies among studies in AVP and OT regulation of pair bonding may be due to the use of different paradigms incorporating different amounts of cohabitation and social stimulation prior to partner preference testing. In addition, icv versus site-specific administration of drugs may result in different concentrations of the drug in a particular brain area to regulate behavior. Therefore, more research is needed to clearly define the extent of sexual dimorphism in the AVP/OT regulation of pair bonding.

3.4. *Brain areas important for AVP/OT regulation of pair bonding*

While the majority of studies concerning neuropeptide regulation of pair bonding have been correlative neuro-anatomical studies or behavioral manipulations induced by icv administration of drugs, recent work has revealed important roles for specific brain nuclei in the neuropeptide regulation of this behavior. As noted above, AVP manipulation in LS regulates partner preference formation in male prairie voles [56]. This result was expected, as prairie vole LS not only contains AVP receptors [48,85,99], but there is also evidence for increased AVP release in LS during mating [6,82]. AVP manipulation in LS also alters male parental care, another social behavior associated with the monogamous life strategy in prairie voles [79]. Further, using the immediate early gene product Fos, as a marker of neuronal activation, increased neural activity is found in LS during mating-induced selective aggression [80]. In other rodents, LS AVP has been demonstrated to be important for memory and individual recognition [33,34]. Together, these data provide strong evidence to support the notion that LS is an important brain area for AVP regulation of pair bonding [56]. Further, OT in the LS also appears to be important for pair bond formation in male prairie voles [56].

Several other brain areas have recently been shown to be involved in AVP/OT regulation of pair bonding. In the ventral pallidum, for example, injections of the V1a receptor antagonist block mating-induced partner preferences [55], whereas overexpression of the V1a receptors facilitates pair bond formation [65]. Additional studies in females have shown that blockade of OT receptors in NAcc prevents pair bond formation [57,96]. As pair bonding is a complex social behavior, it is not surprising that several brain areas and multiple neurochemical systems are involved in the regulation of this behavior.

3.5. *Sex specific effects of pair bonding*

Although recent pharmacological studies have shown that both AVP and OT are involved in pair bonding behavior

in both male and female prairie voles [16,56], sex differences in the regulation of pair bonding, in some cases, appear to be clear. Data have clearly demonstrated that male and female voles differ in the extrahypothalamic AVP pathway, particularly the AVP pathway from BST to LS, and in the activity of this AVP pathway during mating and pair bond formation [6,82]. Males and females also appear to be different in their relative sensitivities to AVP and OT. In a recent developmental study, early postnatal exposure to AVP produces significant and long-lasting increases in aggression in male prairie voles but less effects are found in females [74]. Conversely, neonatal administration of an OT receptor antagonist elevates corticosterone in female, but not male, prairie voles [53]. Furthermore, although central OT manipulation alters partner preference formation in both male and female prairie voles [16], peripheral administration of OT induces partner preferences in females, but not males [20]. There also appear to be robust sex differences in corticosterone regulation of pair bonding—an increase in corticosterone levels induces partner preferences in males but leads to the avoidance of the partner in females [23,24].

There are fewer sex differences in monogamous compared to non-monogamous species [11,73]. This is particularly true for social behaviors such as parental care and pair bonding. It is therefore of interest that while sex differences in the neurochemical systems may often serve to generate sex differences in behavior, it has been suggested that sex specific neurochemical systems, in some cases, may enable males and females to display similar behaviors [25]. This has been suggested to be the case in prairie voles and this topic certainly deserves more attention in further studies.

4. Dopamine regulation of pair bonding

Pair bond formation likely involves multiple types of sensory processing, reward, and memory formation. Given the importance of DA in each of these mechanisms [12,94], we hypothesized that DA would also play an important role in pair bonding. While this hypothesis was first confirmed in early studies in females [42,86], a detailed analysis of DA regulation of partner preference formation has been achieved in our most recent studies using male prairie voles [2,3].

4.1. DA regulates partner preference formation

Similar to studies of the functional roles for AVP and OT in pair bonding, DAergic drugs have been used to either induce or interfere with partner preference formation. For instance, peripheral administration of the non-selective DA receptor antagonist, haloperidol, blocked partner preferences induced by mating, whereas the non-selective DA receptor agonist, apomorphine, induced this behavior in the absence of mating [2]. Since haloperidol treatment did not influence the number of mating bouts, and neither haloperidol nor apomorphine had significant impacts on animals'

locomotor activity in comparison to saline-injected controls, it appears that DA is directly involved in pair bonding.

4.2. NAcc is an important area for DA regulation of pair bonding

While the above data showed that DA is involved in the regulation of pair bonding, subsequent experiments have examined the brain areas important for this DA regulation of pair bonding. NAcc was considered a likely candidate because NAcc DA is important for reward and reinforcement [7]. Importantly, prairie vole NAcc contains DA terminals and receptors [2,41] (Fig. 2A), suggesting that NAcc DA may also regulate these processes in voles. Furthermore, mating has been found to be associated with a 50% increase in the extracellular DA levels in NAcc in female prairie voles [42] and a 33% increase in DA turnover in NAcc in males [2]. These data indicate mating-induced DA release in NAcc, which further suggests the potential importance of NAcc DA in pair bonding, given that mating facilitates pair bond formation.

While NAcc DA involvement in pair bonding was initially shown in females [42], recent studies using male prairie voles have examined, in detail, the nature of NAcc DA regulation of pair bonding [2]. As with peripheral administration, haloperidol injections into NAcc blocked mating-induced partner preferences, indicating that NAcc DA is necessary for pair bond formation (Fig. 2B). In addition, apomorphine injections into NAcc, but not caudate–putamen, induced partner preference formation in the absence of mating (Fig. 2C) [2]. These data, together, support the notion that NAcc DA is critical for pair bond formation in prairie voles [2,42]. It is interesting to note that at low doses, apomorphine administration, either peripherally (0.5 μg) or site-specifically into NAcc (0.04 ng), induced partner preference formation. At high doses, however, apomorphine (5–50 μg for peripheral administration and 4 ng for NAcc administration) did not induce partner preferences [2].

4.3. Opposite modulation of pair bonding by D1- and D2-type receptors

Why does a low, but not high, dose of apomorphine induce pair bonding? The answer seems to involve the existence of two subtypes of DA receptors, namely D1-type and D2-type receptors. Apomorphine binds to both receptors but has a greater affinity for the D2-type, than for the D1-type, receptors [60]. Therefore, it was hypothesized that a low dose of apomorphine induced partner preferences because it activated D2-type receptors preferentially, with minimal activation of D1-type receptors. This notion was supported by data showing that D2-type receptor activation in NAcc by a D2-type specific agonist, quinpirole, induced partner preferences in the absence of mating in male prairie voles [3]. In addition, intra-NAcc blockade of D1-type receptors in females did not inhibit mating-induced partner

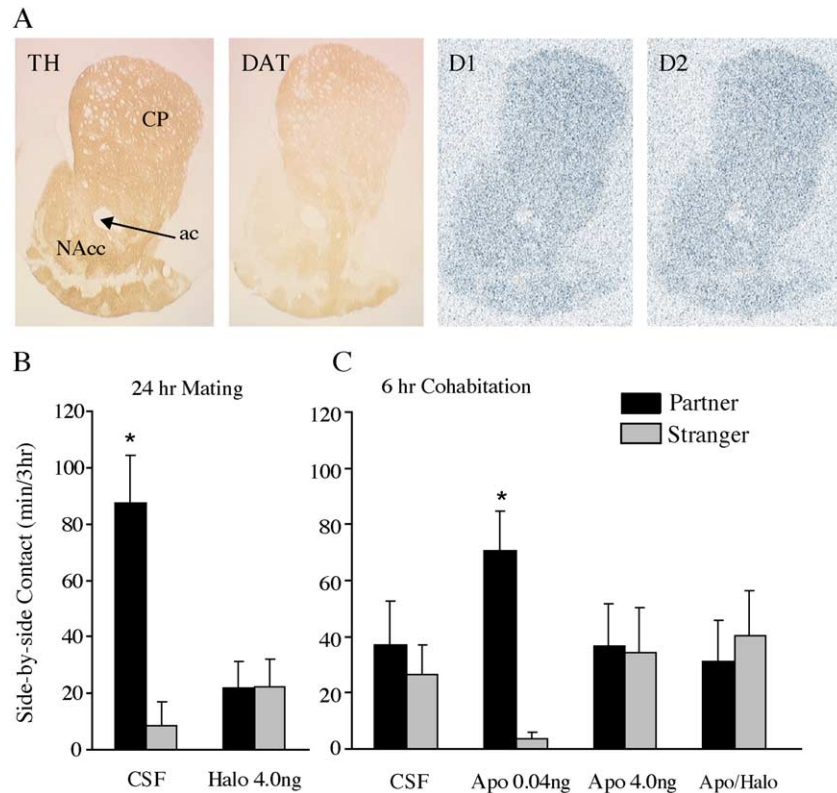


Fig. 2. Nucleus accumbens dopamine is important for pair bond formation in male prairie voles. (A) Photomicrographs displaying immunoreactive staining for tyrosine hydroxylase (TH) and dopamine transporter (DAT) as well as autoradiographic binding for dopamine D1-type (D1) and D2-type (D2) receptors in brain sections from male prairie voles. AC: anterior commissure, CP: caudate–putamen, and NAcc: nucleus accumbens. (B) Twenty four hours of mating induces partner preference formation in control males receiving intra-NAcc injections of CSF, but not in males injected with the DA receptor antagonist, haloperidol (Halo). (C) Control males (CSF) do not show partner preferences following 6 h of cohabitation with a female without mating. However, males receiving the low (0.04 ng), but not the high (4.0 ng), dose of the dopamine receptor agonist, apomorphine (Apo), into NAcc display more side-by-side contact with the partner, and this induced partner preference formation is blocked by co-administration of haloperidol (0.4 ng). Error bars indicate standard errors of the means. * $p < 0.05$. Data are adapted from Ref. [2].

preference formation [42]. Therefore, data from both male and female prairie voles suggest that pair bond formation is mediated by D2-type DA receptors [3,42,86].

At high doses, apomorphine also activates D1-type receptors, and it was hypothesized that this activation prevents pair bond formation. This was tested directly by using the D1-type specific agonist, SKF 38393. As noted above, male voles injected with quinpirole into NAcc showed partner preference formation. However, males injected with a combination of quinpirole and SKF 38393 did not display this behavior, indicating that D1 receptor activation blocked pharmacologically induced partner preference formation. In addition, activation of D1-type receptors in NAcc also prevented partner preferences induced by 24 h of mating. These data strongly suggest that activation of D1- and D2-type receptors exerts opposite modulation over pair bond formation [3].

Knowledge of DA receptor function with respect to pair bonding has prompted investigation of DA receptor expression between prairie voles with different social experience. Specifically, given that pair bond formation is a stable and enduring behavioral alteration, it was hypothesized that pair bonded animals may display DA receptor levels in NAcc that

would facilitate stability of the bond. Our recent data indicate that in male prairie voles, the density of D1-type receptors in NAcc did not change following 24 h of ad libitum mating but did show a significant increase two weeks after being paired with a female [B.J. Aragona and Z. Wang, unpublished data]. As this cohabitation resulted in successful impregnation, it was likely that these animals were pair bonded. This change in D1-type receptors was found in NAcc but not in other DAergic brain areas such as the caudate–putamen. As activation of D1-type receptors is antagonistic to pair bond formation, this neural modification may prevent the formation of new pair bonds. This would, therefore, promote the maintenance of the already formed bond and, thus, the stability of a monogamous social organization.

4.4. Intracellular mechanisms of DA regulation of pair bonding

The opposite modulation of D1- and D2-type receptors of pair bond formation may lie in the activation of specific intracellular mechanisms. All DA receptors are seven transmembrane domain G-protein coupled receptors. D1-type receptors activate stimulatory G-proteins and their

activation increases intracellular cAMP, whereas D2-type receptors are coupled to inhibitory G-proteins that decrease cAMP [60]. Given the opposite modulation of the cAMP second messenger system by D1- and D2-type receptor activation and that partner preferences can be induced by D2-type receptor stimulation, we hypothesized that NAcc DA may facilitate partner preference formation by down regulation of the cAMP cascade. This notion is supported by our pilot data showing that inactivation of inhibitory G-proteins, via site-specific administration of pertussis toxin into NAcc, prevents mating induced partner preferences. Further, activation of inhibitory G-proteins eventually decreases intracellular levels of cAMP, leading to a decrease in protein kinase A (PKA) activity. We have also found that competitive inhibition of PKA with Rp-cAMPS administered directly into NAcc induced partner preference formation in male prairie voles [B.J. Aragona and Z. Wang, unpublished data]. Together, these data further demonstrate that DA regulates pair bond formation within NAcc via activation of D2-type receptors and subsequent down regulation of the cAMP second messenger system. In ongoing experiments, we are measuring the effects of mating or D1/D2 receptor manipulation on cAMP levels in the prairie vole NAcc. Interestingly, this DAergic modulation of pair bonding is similar to NAcc DA regulation of drug seeking behavior [69–72]. These data suggest that social and drug reward may act on the same neural mechanism.

5. Neurochemical interactions in the regulation of pair bonding

It is not surprising that complex social behaviors, such as pair bonding, are under the control of multiple neurochemical systems. Instead of acting independently, these neurochemicals may interact with each other in the

regulation of pair bonding. At present, relatively few studies have been performed addressing such interactions; however, these studies have shown that AVP, OT, and DA indeed interact in the regulation of pair bonding.

5.1. AVP–OT interactions in pair bonding

Central AVP and OT interactions were examined in both male and female prairie voles [16]. Administration (icv) of AVP or OT induced partner preference formation after only 1 h of cohabitation. Importantly, pretreatment with either the V1a receptor antagonist or OT receptor antagonist inhibited AVP- or OT-induced partner preferences. These data indicate that AVP and OT interact to regulate pair bond formation. In a recent study in male prairie voles, administration of the V1a antagonist or OT receptor antagonist directly into LS blocked mating-induced partner preferences, and AVP-induced pair bonding was blocked by concurrent administration of either the V1a antagonist or OT receptor antagonist [56] (Fig. 3). This finding demonstrates that interactions of AVP and OT in LS are important in partner preference formation in male prairie voles.

5.2. DA–OT interactions in pair bonding

In the above, we suggested that mating-induced DA release in NAcc is important for pair bonding. However, mating also induces DA release in NAcc in other species of rodents that do not form pair bonds [8,22,64], and mating induces DA release in the caudate–putamen similarly in both monogamous and non-monogamous voles [19]. Therefore, mating-induced DA release is unlikely to be fully responsible for pair bonding seen in monogamous voles. Instead, DA involvement in pair bonding may be due to unique interactions with other

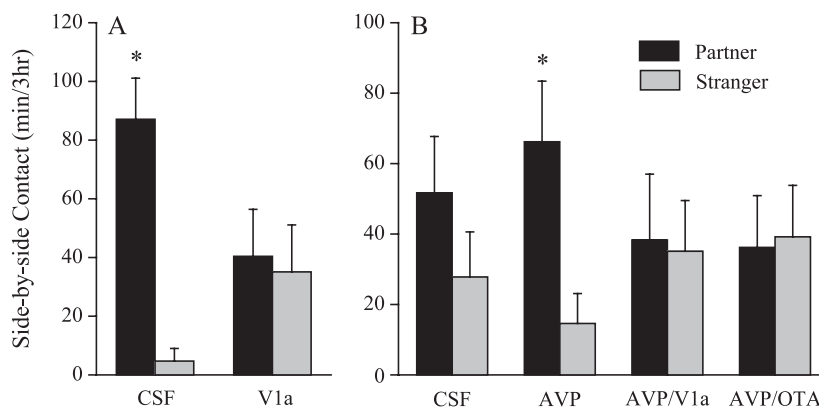


Fig. 3. Manipulation of vasopressin (AVP) and oxytocin (OT) in the lateral septum (LS) alters partner preference formation in male prairie voles. (A) Twenty four hours of mating induces partner preference formation in control males receiving intra-LS injections of CSF but not in males receiving injections of the AVP V1a receptor antagonist (V1a, 0.5 ng). (B) Control males (CSF) do not show partner preferences following 6 h of cohabitation with a female without mating. However, males dialyzed with AVP (0.8 ng/ μ m/min) into LS display partner preference formation, and this AVP-induced behavior is blocked by co-administration of the same dose of V1a or the OT receptor antagonist. Error bars indicate standard errors of the means. * $p < 0.05$. Data are adapted from Ref. [56].

neurochemical systems that differ between monogamous and non-monogamous voles. Neuroanatomical studies indicated that prairie voles have more OT receptors in NAcc compared to those found in non-monogamous voles [47]. Although not yet examined in male prairie voles, in females, intra-NAcc administration of an OT receptor antagonist blocked partner preferences induced by D2-type receptor activation [57,96]. Furthermore, blockade of D2-type receptors in NAcc prevents partner preferences induced by either quipirole or OT [57]. These data indicate that concurrent activation of OT and D2-type receptors and interactions of the two systems in NAcc are necessary for pair bond formation. In the same study, as expected, a D1-type antagonist did not block partner preferences induced by OT.

Finally, the ventral pallidum is an area that receives the major output from NAcc [43], and contains a high density of the V1a receptors [48,85] that have been implicated in pair bonding in male prairie voles [55,65]. Given the large degree of interconnections between NAcc and ventral pallidum, it is possible that DA and AVP systems interact to influence pair bond formation. This needs to be examined in further studies. (After this manuscript was submitted, a newly published study by Lim MM and her colleagues (*Nature* 2004;429:754-757) indeed demonstrated that AVP and DA interact in the ventral pallidum to regulate pair bonding behavior).

6. Conclusion

In summary, the prairie vole model provides an excellent opportunity to study the neurobiology of pair bonding. Recent research has demonstrated that AVP, OT, and DA interact in the regulation of this extremely complex behavior. Monogamous prairie voles possess species specific patterns of neurochemical systems (such as central AVP or OT receptor distributions) that may contribute to pair bond formation. In addition, it has been demonstrated that pair bonding is regulated, in part, by the same neural pathway, such as NAcc DA, that also mediates drug reward and addiction [7]. These data provide evidence to support the contention that drug addiction is a disorder associated with maladaptive access to natural reward systems [32,51,93,100], and, therefore, the prairie vole provides a potential model to examine more closely the involvement of reward processing on social behavior. Studies of pair bonding in prairie voles have also provided valuable information regarding the formation and regulation of complex social bonds. Such information is of potential importance for human health, as humans form similar social bonds, and the inability to form social bonds is associated with several psychological disorders [76]. It is hoped that future research focusing on pair bonding in the prairie vole will lead to a better understanding of normal and abnormal social behavior in humans.

Acknowledgements

We thank Drs. J. Thomas Curtis and Yan Liu as well as Christie D. Fowler and Michael Smeltzer for their critical reading of the manuscript. We also thank Drs. Elaine Hull, Lique Collen, and Stephen Woods for their helpful comments and suggestions. This work was supported by National Institutes of Health grants MH-54554, MH-58616 and MH-66734 (ZXW) and MH-67396 (BJA).

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