The Prairie Vole (*Microtus ochrogaster*): An Animal Model for Behavioral Neuroendocrine Research on Pair Bonding

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Abstract

Pair bond formation has been investigated much less than many other social behaviors, perhaps in part because traditional laboratory mice and rats do not exhibit this behavior. However, pair bonding is common among monogamous animals such as the prairie vole (*Microtus ochrogaster)*. In this review, we discuss how the prairie vole has been used as a model system to investigate the neurobiology of pair bonding. Descriptions include neuroanatomical differences between monogamous and non-monogamous voles, as well as how manipulations of vasopressin, oxytocin, dopamine, and corticosterone systems affect pair bond formation. Also summarized are potential interactions among these systems that regulate pair bonding, and the extent of sexual dimorphism in underlying mechanisms. Pair bonding in prairie voles is an excellent model system for studying central processing of social information. Understanding the mechanisms underlying this behavior may provide important insights into human disorders associated with impaired social functioning.

Key Words: corticosterone; dopamine; mating; *Microtus;* monogamy; oxytocin; pair bond; social attachment; vasopressin

Introduction

Behavioral neuroscience research has been conducted primarily on typical laboratory rodents, including mice and rats, which display stereotypic social behaviors associated with reproduction, maternal care, and aggression. The reliable expression of such behaviors has made it possible to examine their underlying neural mechanisms (for reviews see Nelson and Chiavegatto 2001; Pfaus et al. 2001; Rissman et al. 1999; Stern and Lonstein 2001). However, these traditional laboratory animals do not display certain social behaviors, such as pair bond formation. A pair bond is defined as a stable relationship between members of a breeding pair that share common territory and parental duties. Analogous social bonds are formed by humans, and the inability to form such bonds is a key diagnostic component in certain psychological disorders (Volkmar 2001). Given that traditional laboratory rodents do not form pair bonds, an alternative animal model is needed to study this behavior. Recently, utilization of a nontraditional laboratory rodent, the monogamous prairie vole (*Microtus ochrogaster*), has generated valuable data regarding the neurobiology of pair bonding. We first introduce the prairie vole model and then review recent research examining neurochemical regulation of pair bonding. We also briefly discuss future directions for research using voles. It is important to note that although this review focuses on pair bonding, voles are also useful models for other topics of behavioral neuroscience research, such as activity rhythms, paternal behavior, and estrous induction (Carter et al. 1989; Gerkema and van der Leest 1991; Lonstein and De Vries 2000).

Prairie Vole Model

Prairie voles are small brown rodents (about 40 g) distributed primarily in the grasslands of the central United States (Hall 1981; Tamarin 1985). In these environments, prairie voles have adapted to scarce water supplies and food sources of minimal caloric value (Birney et al. 1976; Getz 1978; McGuire et al. 1993; Tamarin 1985). It has been suggested that monogamy is selected for under conditions of limited resources because two parents may be necessary to care for and protect dependent young (Emlen and Oring 1977), and it has been speculated that this case applies to prairie voles (Carter et al. 1995; Wang and Novak 1992). In the field, the majority of prairie vole nests are occupied by a pair bonded male and female along with their offspring (Getz and Hofmann 1986; Getz et al. 1981, 1993), and such a breeding pair typically remains together until one dies (Getz and Carter 1996). Another behavior indicative of monogamy is male parental behavior. Unlike most other rodents, prairie vole males contribute significantly to nest building, nest guarding, and other parental behaviors such as huddling and retrieving pups that wander from the nest (Getz and Carter 1996; Gruder-Adams and Getz 1985; Thomas and Birney 1979).

Interest in the monogamous behaviors observed in the field has prompted investigators to bring prairie voles into the laboratory. Voles breed easily in captivity, and laboratory maintenance is comparable with that of other rodents (Ranson 2003). Monogamous behaviors, similar to those

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observed in nature, are also reliably expressed under laboratory conditions. For instance, prairie voles mate preferentially with one partner, remain together during gestation, and display biparental care throughout lactation (Carter and Getz 1993; Dewsbury 1987; Getz and Carter 1996; McGuire and Novak 1984; Oliveras and Novak 1986; Thomas and Birney 1979). Pair bonding is studied in the laboratory by examining stereotypic behaviors that are necessary for the formation of the bond. Specifically, pair bonded animals must recognize and choose their mate over unfamiliar conspecifics, and they must even aggressively reject unfamiliar conspecifics from their territory. Indeed, prairie voles prefer to be with their mate, indicated by significantly more time spent with their mate (partner) versus a conspecific stranger in a subsequent choice test after mating or extensive cohabitation (Williams et al. 1992). This behavior is referred to as a partner preference. Pairings that induce partner preference formation also induce an increase in aggressive behavior toward unfamiliar conspecifics (selective aggression) (Bowler et al. 2002; Wang et al. 1997a; Winslow et al. 1993), and this behavior serves to guard mate and territory (Carter and Getz 1993). Partner preferences and selective aggression are thus used as behavioral indices for pair bond formation.

Herein we describe the method used in our laboratory to assess partner preference formation. This test was first developed in Dr. Sue Carter's laboratory (Williams et al. 1992), and it has been adapted by several others (Young et al. 1998). Although specific paradigms may differ across laboratories, the general concept is the same. The testing apparatus consists of a central chamber with tubes connecting it with two identical chambers, one containing the partner and the other a conspecific stranger. These two stimulus animals are tethered in their chambers and thus do not interact with each other, whereas the subject is free to move throughout the testing apparatus during the 3-hr partner preference test. A customized computer program using a series of light beams across the connecting tubes monitors subject movement between the cages and time spent in each cage (Figure 1). Pair bonding is inferred when subjects spend significantly more time in contact with their partners than with strangers.

It has been demonstrated that in prairie voles, 24 hr of ad libitum mating reliably induces partner preference formation (Insel and Hulihan 1995; Winslow et al. 1993; see Figure 1), and thus this paradigm has been used to investigate the neurochemical mechanisms underlying pair bonding. For instance, drugs can be administered before pairing the subject with its partner, and if drug manipulation prevents mating-induced partner preference formation, then the neurochemical system modulated by the drug is implicated in pair bonding. Furthermore, after 6 hr of cohabitation, in the absence of mating, prairie voles are equally likely to spend time in contact with either the partner or a stranger (i.e., fail to show a partner preference; Williams et al. 1992). Because this manipulation reliably fails to produce partner

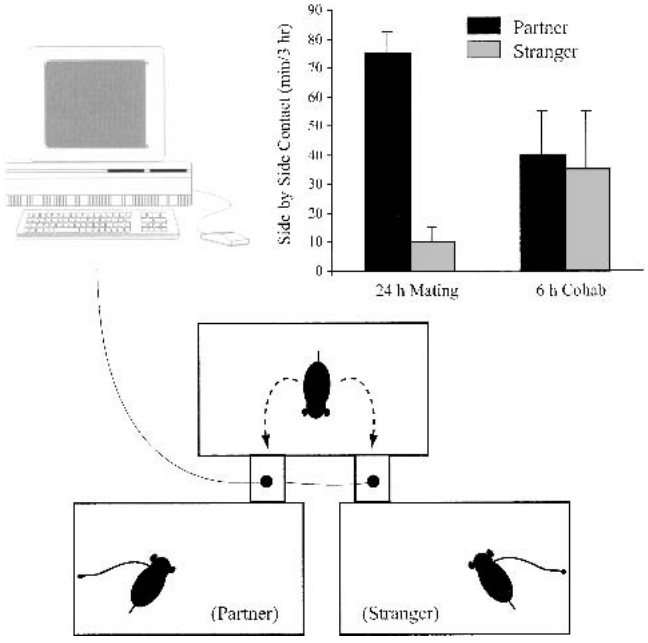

Figure 1 (Bottom center) Apparatus used to perform partner preference tests in our laboratory. Each cage is identical (20 × 25 × 45 cm), and hollow tubes (7.5 ×16 cm) connect the neutral cage to those containing stimulus animals. (Upper left) Infrared sensors record movement of the subject, and these data are automatically recorded. The primary behavior of interest is side-by-side contact time or huddling. An experimenter blind to treatment watches time lapse video of the partner preference test and records contact duration. (Upper right) Idealized data of side-by-side contact during the 3-hr partner preference test. A *t*-test reveals that 24 hr of ad libitum mating before the partner preference test leads to the subject spending significantly more time in contact with the partner compared with the stranger. In contrast, 6-hr cohabitation in the absence of mating results in subjects spending approximately equal time, on average, with either the partner or the stranger.

preferences, it has been used to assess whether drug manipulations can induce partner preferences.

It is important to note that although mating facilitates partner preference formation (Williams et al. 1992), this behavior is also induced under certain circumstances in the absence of mating. For example, extended cohabitation (24-48 hr) with a male induces partner preference formation in female prairie voles (Williams et al. 1992). However, some reports suggest that even shorter cohabitation periods can induce partner preferences in subjects that were previously isolated (DeVries et al. 1995, 1996). Additionally, an environmental manipulation (e.g., forced swimming), which induces an increase in hypothalamic-pituitary-adrenal axis activity in voles (DeVries et al. 1996; Liu et al. 2001a), results in partner preference formation in male prairie voles after only 1 hr of cohabitation with a female (DeVries et al. 1996). For females, an extended cohabitation even induces preferences for same sex individuals (DeVries et al. 1997). Together, these data indicate that partner preference forma-

tion is likely a very complicated physiological and behavioral process that is influenced by a variety of endogenous and exogenous factors. In this review, we initially focus on partner preferences induced or facilitated by mating, and then discuss partner preferences formed by females that likely precede mating.

Comparative studies using different vole species have also proven to be valuable in addressing the neurobiology of social behavior. Montane (*Microtus montanus*) and meadow voles (*Microtus pennslyvanicus*) are taxonomically close to prairie voles but have non-monogamous life strategies and are asocial. It has been demonstrated that compared with prairie voles, non-monogamous voles show low levels of social affiliation, do not mate preferentially with one partner, exhibit no partner preferences after mating, and females alone provide parental care after parturition (Dewsbury 1987; Insel and Hulihan 1995; Jannett 1982; McGuire and Novak 1984). Importantly, non-monogamous and monogamous voles show similar patterns in nonsocial behaviors (Tamarin 1985), thus providing tremendous potential for comparative studies on their social behaviors.

It is worth noting that differences have also been found between voles of the same species from different geographic locations. For example, the background information for prairie voles described above is based on animals trapped from Illinois. Although prairie voles from Kansas show robust partner preference formation, they differ in sexual dimorphism, parental behavior, and responsiveness to exogenous estrogen and vasopressin in comparison with their counterparts from Illinois (Cushing et al. 2001; Roberts et al. 1998a,b). In this article, we focus on prairie voles originally trapped in Illinois because they have been used in the majority of studies examining neurochemical regulation of pair bonding.

Neuropeptide Regulation of Pair Bonding

Early studies on the neurobiological basis of pair bond formation primarily focused on the neuropeptides arginine vasopressin (AVP[1]) and oxytocin (OT[1]). An important factor in choosing AVP and OT was that these peptides were known to be involved in another type of social bond, the bond between mother and offspring. For example, central administration of AVP or OT increased maternal behavior (Insel and Harbaugh 1989; Kendrick et al. 1987; Pedersen and Prange 1979; Pedersen et al. 1982), and OT also decreased isolation-induced distress calls in rat pups (Winslow and Insel 1991). Furthermore, AVP and OT were known to be involved in reproductive behavior (Argiolas et al. 1988, 1989; Carter 1992; Insel 1992), which is important because mating facilitates pair bond formation in prairie voles (Insel and Hulihan 1995; Williams et al. 1992; Winslow et al. 1993). Additionally, AVP plays an important role in the regulation of aggression (Albers et al. 1992; Compaan et al. 1993; Ferris and Delville 1994; Ferris et al. 1997), and selective aggression is also indicative of pair bonding (Bowler et al. 2002; Wang et al. 1997a; Winslow et al. 1993).

Neuroanatomical Correlates

Comparative studies have been performed on AVP and OT systems between vole species with different life strategy and social behavior. These studies addressed three questions regarding central AVP and OT systems: (1) Do monogamous and non-monogamous voles differ; (2) do males and females within the monogamous voles differ; and (3) does affiliative behavior differentially alter neuropeptide systems either between species or between sexes within the same species?

The neuroanatomical studies performed in voles have been reviewed in detail (De Vries and Miller 1998; Wang et al. 1998; Young et al. 1998); herein we briefly summarize selected data from comparative studies. Immunocytochemistry and in situ hybridization have been used to examine AVP- and OT-producing cells and their projections in a variety of vole species (Bamshad et al. 1993; Wang 1995; Wang et al. 1996). Similar to other rodents, AVP-positive cells are found in hypothalamic nuclei as well as in the bed nucleus of the stria terminalis (BNST[1]) and the medial nucleus of the amygdala (MeA[1]). Dense labeling of AVP immunoreactive (AVP-ir) fibers is found in the lateral septum (LS[1]), lateral habenular nucleus (LH[1]), diagonal band, medial preoptic area (MPOA[1]), BNST, and MeA. OT-ir cells are also found in hypothalamic nuclei and in other brain areas including BNST, MPOA, and lateral hypothalamus (Wang et al. 1996). Some species differences have been observed. For example, prairie voles have fewer OT-positive cells in the MPOA and BNST, but a higher density of AVP-ir fibers in the LS, than do non-monogamous voles (Wang 1995; Wang et al. 1996). In general, however, the morphology and distribution pattern of central AVP/OT systems are similar across vole species (Wang et al. 1996).

A sexually dimorphic pattern is evident for AVP pathways in the vole brain. Across species, male voles have more AVP-ir or AVP mRNA-labeled cells in the BNST and MeA, and a greater density of AVP-ir fibers in LS and LH compared with females (Bamshad et al. 1993; Wang 1995; Wang et al. 1996). This AVP pathway is also found to be gonadal steroid dependent: castration reduces the number of AVP-ir cells and the density of AVP-ir fibers, whereas testosterone replacement reverses this effect (Wang and De Vries 1993). This sexually dimorphic and steroid-dependent AVP pathway resembles those reported in other species of rodents (Crenshaw et al. 1992; van Leeuwen et al. 1985).

[1]Abbreviations used in this article: AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; CORT, corticosterone; DA, dopamine; icv, intracerebroventricular; LH, lateral habenular nucleus; LS, lateral septum; MeA, medial amygdale; MPOA, medial preoptic area; NAcc, nucleus accumbens; OT, oxytocin; OTA, oxytocin antagonist; OVX, ovariectomized; V1a, vasopressin V1a receptor; VMH, ventromedial hypothalamus.

Interestingly, experience with the partner and mating exert sex-specific effects on this AVP pathway in prairie voles. Three days of cohabitation with an opposite sex individual (which typically induces mating) significantly decreases the density of AVP-ir fibers in the LS and LH and increases the level of AVP mRNA labeling in the BNST in male, but not female, prairie voles (Bamshad et al. 1994; Wang et al. 1994b). This effect is also species specific, in that no group differences are found in the level of AVP mRNA labeling in the BNST of non-monogamous voles (Wang et al. 1994b). In rats, AVP-producing cells in the BNST project to the LS (De Vries et al. 1983). Given the increase in AVP mRNA expression in the BNST and a decrease in AVP-ir staining in the LS after mating, it has been suggested that mating induces AVP release in the LS of male monogamous, but not non-monogamous, voles (Wang et al. 1998). There is also evidence that mating induces central OT release in mammals (Carter 1992; Jirikowski 1992); however, direct release of neuropeptides has not been tested in voles.

Voles with different life strategies and social behaviors may also differ in brain responsiveness to released neuropeptides. This notion has been examined by comparing central AVP/OT receptors between vole species. There are three known subtypes of AVP receptors, and in the brain, AVP acts primarily on the vasopressin V1a receptors (V1A[1]). Monogamous and non-monogamous voles show remarkable differences in the distribution pattern and regional density of the V1a receptors, measured by receptor binding and mRNA labeling (Insel et al. 1994; Wang et al. 1997b; Young et al. 1997). For example, prairie voles have more V1a receptors in the ventral pallidum, BNST, and thalamus; whereas montane voles have more in the LS and ventromedial hypothalamus (VMH[1]). Differences have also been found in OT receptors; prairie voles show more in the nucleus accumbens (NAcc[1]), prelimbic cortex, BNST, lateral amygdala, and anterior olfactory nucleus, whereas montane voles have more in LS, VMH, and central and posterior cortical amygdala (Insel and Shapiro 1992; Witt et al. 1991; Young et al. 1996). Some of these differences in the V1a and OT receptors are present at birth, and others change over the course of postnatal development (Wang and Young 1997; Wang et al. 1997b). These differences in receptor distribution do not generalize to all peptides because monogamous and non-monogamous voles do not differ in benzodiazepine or μ-opioid receptor distribution (Insel and Shapiro 1992).

Studies have also been performed that have assessed receptor changes induced by social behavior. OT receptor binding is elevated in the anterior olfactory nucleus of female prairie voles by exposure to male chemosensory cues (Witt et al. 1991). Similar increases were found in the lateral amygdala of female montane voles and VMH of both female prairie and montane voles within 24 hr of parturition (Insel and Shapiro 1992; Wang et al. 2000). These findings indicate that social behavior can affect OT receptors. However, mating does not alter AVP receptor distribution in voles (Wang et al. 2000).

Evidence for the functional significance of neuropeptide receptor distribution comes from a study focusing on the V1a receptors. Prairie and montane voles have the same V1a receptor gene; however, prairie voles have an additional ≈400 bp insert in the promoter region (Young et al. 1999). Transgenic mice were created that had a prairie vole V1a receptor promoter region inserted, and these mice subsequently expressed V1a receptors in a distribution pattern similar to that of prairie voles. In addition, these mice also exhibited altered responses to central administration of AVP, such as increases in affiliative olfactory investigation and grooming (Young et al. 1999). These data suggest that a particular distribution pattern of neuropeptide receptors may be important for these and other affiliative behaviors.

Direct Neuropharmacological Testing

Although neuroanatomical studies implicate AVP/OT involvement in social behavior, such data are correlative. For this reason, direct involvement of AVP/OT in pair bonding was also tested experimentally, by neuropharmacological manipulation. In an early study that addressed pair bonding in male prairie voles (Winslow et al. 1993), subjects paired with estrogen-primed females for 24 hr with ad libitum mating displayed partner preferences and selective aggression—behaviors associated with pair bonding. Males paired with ovariectomized (OVX[1]) females in the absence of mating did not show either behavior. However, if males paired with non-estrogen-primed females received continuous intracerebroventricular (icv[1]) infusion of AVP, but not OT, then these animals showed partner preference formation in the absence of mating. Additionally, acute administration (icv) of the V1a receptor antagonist, but not an OT receptor antagonist (OTA[1]), blocked mating-induced partner preferences. In the same study, AVP, but not OT, infusions also induced selective aggression toward a conspecific stranger in male prairie voles in the absence of mating, whereas the V1a receptor antagonist, but not OTA, blocked mating-induced selective aggression. Importantly, AVP and the V1a receptor antagonist manipulations neither influenced mating nor altered locomotor activity, indicating the specificity of drug effects to pair bonding behavior. Together, these data suggest that central AVP, but not OT, plays an important role in the regulation of pair bonding in male prairie voles.

OT involvement in pair bond formation was first demonstrated in female prairie voles (Insel and Hulihan 1995; Williams et al. 1994). OVX females paired with a male for 6 hr in the absence of mating did not show partner preferences. However, if females received icv infusions of OT, but not AVP, at 0.5 ng/hr before and continuously during the cohabitation with a male, they displayed partner preferences. Similar to males, 24 hr of mating also induced part-

ner preference formation in female prairie voles, and this behavior was blocked by icv administration of OTA, but not the V1a receptor antagonist. These data suggest that OT, but not AVP, is involved in the regulation of pair bonding in female prairie voles (Insel and Hulihan 1995).

Although these early data indicate a sexually dimorphic mechanism—AVP involvement in the regulation of pair bonding in males and OT involvement in the same behavior in females—recent studies suggest that AVP/OT regulation of pair bonding may be more complex. In a study in which a wider range of doses of AVP and OT was used (Cho et al. 1999), icv injections of either AVP or OT at 100-ng dosage induced partner preferences in female prairie voles that were housed with a male for 1 hr in the absence of mating. In addition, icv injections of AVP (1-100 ng) or OT (10-100 ng) induced partner preferences in male prairie voles when the same paradigm was used. These data indicate that both AVP and OT are involved in the regulation of pair bonding in both male and female prairie voles. This notion is further supported by recent data from our laboratory. Administration of either the V1a receptor antagonist or OTA into LS blocked mating-induced partner preferences in male prairie voles (Liu et al. 2001b); and central infusions (icv) of AVP at a higher dose (2.5 ng/hr), relative to that used in a previous study (Insel and Hulihan 1995), induced pair bonding in female prairie voles (Liu and Wang, unpublished data).

Several factors might have contributed to the discrepancies among the experiments described above. In the study in which AVP did not affect pair bonding in female prairie voles, only a single dose of AVP and the V1a receptor antagonist was tested (Insel and Hulihan 1995). In contrast, a more detailed dose response was performed to reveal a possible role for AVP in female pair bond formation (Cho et al. 1999), although one cannot exclude the possibility that high doses of AVP also acted on OT receptors (Barberis and Tribollet 1996). Furthermore, OT manipulation did not alter male behavior when delivered into the ventricular system (Winslow et al. 1993); however, site-specific administration of OTA into LS blocked partner preference formation in male voles (Liu et al. 2001b). It is possible that icv administration did not result in sufficient drug concentrations acting in particular brain areas. An additional complication is that different paradigms incorporating different amounts of cohabitation and social stimulation have been used across studies (Cho et al. 1999; Insel and Hulihan 1995; Winslow et al. 1993). Therefore, systematic and detailed studies comparing males and females are needed to understand the true extent of potential sex differences. It is worth noting that despite these discrepancies, sex differences in the regulation of pair bonding, in some cases, appear to be clear. There are differences between male and female voles in their relative sensitivities to AVP, OT, and dopamine (DA^1) (see below). There also appear to be robust sex differences in glucocorticoid regulation of pair bonding (DeVries et al. 1996). In addition, sex differences may exist in other systems that interact with AVP and OT to regulate pair bonding.

Site Specificity of Pair Bonding

Which brain areas are involved in pair bonding? In an initial attempt to examine neuronal activation associated with pair bond formation, an immediate early gene product, *c-fos,* was used to label brain areas activated during mating-induced selective aggression in male prairie voles (Wang et al. 1997a). Recently, the same approach was also used to map neuronal activation in the vole brain after exposure to same- or opposite-sex individuals (Cushing et al. 2003) or after a period of ad libitum mating conducive for pair bonding (Curtis and Wang 2003). Increased *c-fos* labeling was found in BNST, MeA, and LS, which implicates these areas in pair bond formation. Interestingly, these brain areas contain AVP-producing cells, and these cells and their projections are altered during mating and partner preference formation (Bamshad et al. 1993; Wang et al. 1994b). In a recent study, administration of AVP (via reverse dialysis) into LS induced partner preference formation, whereas injections of the V1a receptor antagonist blocked this behavior induced by mating or by AVP administration (Figure 2; Liu et al. 2001b). In the same study, OTA injections in LS also blocked mating- or AVP-induced partner preferences. Together, these data suggest that LS is a brain area in which AVP and OT regulate pair bond formation in male prairie voles.

Manipulations of LS AVP receptors also alters parental behavior in males. Injections of AVP directly into LS enhanced, whereas the V1a antagonist reduced, paternal behavior (Wang et al. 1994a). Lesions of the MeA in males also reduced paternal as well as other affiliative behaviors (Kirkpatrick et al. 1994). Although the BNST has not been

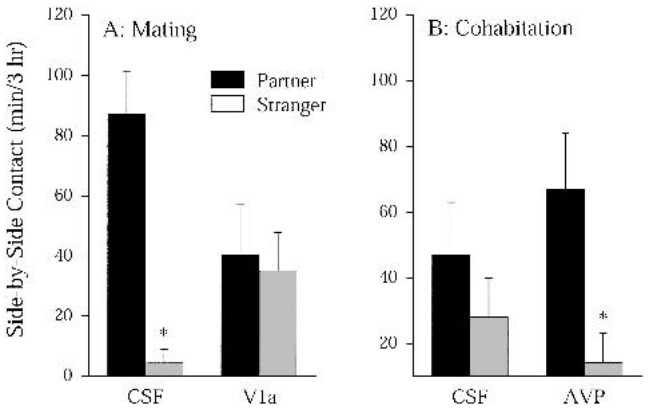

Figure 2 Vasopressin in the lateral septum (LS) is important for pair bonding in male prairie voles. (A) Male prairie voles that mated ad libitum for 24 hr spent significantly more contact time with their partners compared with the strangers; however, administration of an arginine vasopressin (AVP) V1a receptor antagonist into the LS blocked mating-induced pair bonding. (B) Males that cohabited with females for 6 hr in the absence of mating did not show partner preferences; however, continuous administration of AVP into the LS induced this behavior in the absence of mating. CSF, cerebrospinal fluid.

tested directly, it is highly interconnected with the MeA and LS (Newman 1999) and is implicated in mating-induced AVP release in prairie voles (Wang et al. 1998). The involvement of these brain regions in pair bonding is consistent with the critical role they play in the regulation of social behavior in other species (Newman 1999).

Several brain areas implicated in reward learning, such as NAcc and ventral pallidum (Ikemoto and Panksepp 1999; Kretschmer 2000), also contain high levels of OT or V1a receptors (Wang et al. 1997b; Young et al. 2001). Intra-NAcc injections of OTA blocked mating-induced partner preferences (Young et al. 2001), whereas injections of OT induced this behavior in the absence of mating in female prairie voles (Liu and Wang 2003). AVP in the ventral pallidum is also important for pair bond formation in male prairie voles. Injections of the V1a receptor antagonist into the ventral pallidum blocked mating-induced partner preferences (Lim and Young 2002), whereas overexpression of V1a receptors in this area (via adeno-associated viral vector insertion) facilitated pair bond formation (Pitkow et al. 2001).

Dopamine Regulation of Pair Bonding

It has been demonstrated convincingly that mating induces DA release in a variety of rodent species, including the prairie vole (Curtis et al. 2003; Gingrich et al. 2000; Mas et al. 1995; Pfaus et al. 1990; Robinson et al. 2001). Given that mating facilitates partner preference formation, it was hypothesized that mating-induced DA release is involved in the regulation of pair bonding. This hypothesis has been strongly supported by behavioral pharmacological studies. After 6 hr of cohabitation in the absence of mating, female prairie voles that were treated peripherally with a nonselective DA agonist, apomorphine, but not vehicle alone, displayed partner preferences, whereas administration of a nonselective DA antagonist, haloperidol, blocked this behavior induced by mating (Wang et al. 1999). DA involvement in pair bonding has also been demonstrated in male prairie voles using a similar experimental paradigm (Aragona et al. 2003a). For males, however, lower doses of apomorphine were required for partner preference formation compared with females; and high doses of apomorphine (i.e., 50 μg), which were effective in females, failed to induce pair bonding in males (Aragona et al. 2003a). These data indicate that male and female prairie voles differ in responsiveness to DA drugs, and that males are more sensitive.

A brain region believed to be involved in DA-regulated reward processing is the NAcc (Ikemoto and Panksepp 1999; Koob and Nestler 1997; Schultz 1997; Self and Nestler 1998; Wise 1996). As with other rodents, prairie vole NAcc reveals dense labeling of DA terminals and receptors (Aragona et al. 2003a,b). In males, intra-NAcc administration of haloperidol blocked mating-induced partner preferences, whereas low, but not high, doses of apomorphine induced this behavior in the absence of mating (Aragona et al. 2003a).

It is of interest that low doses of apomorphine are required to induce partner preferences in males. There are two different families of DA receptors (D1- and D2-type), and apomorphine preferentially binds to D2-type receptors (Missale et al. 1998). Therefore, low doses of apomorphine may activate primarily D2-type receptors. This possibility suggests that DA acts via D2-type receptors to regulate pair bonding. This notion is supported by studies using female prairie voles in which peripheral or intra-NAcc administration of the D2-type agonist quinpirole, but not the D1-type agonist SKF 38393, induced partner preferences in the absence of mating, . Similarly, administration of the D2-type antagonist eticlopride, but not the D1-type antagonist SCH 23390, blocked mating-induced partner preferences (Gingrich et al. 2000; Wang et al. 1999). Furthermore, a recent study in male prairie voles not only confirmed the observation that activation of D2-type receptors in NAcc is important for partner preference formation but also extended this finding by demonstrating that D2-type receptors in the shell, but not the core, of NAcc are involved in pair bond formation (Aragona et al. 2003b). Additionally, administration of quinpirole, but not a combination of quinpirole and the D1-type receptor agonist SKF 38393, into NAcc induced partner preferences (Aragona et al. 2003b). This finding suggests that D1-type receptors are not simply uninvolved in pair bond formation but, rather, their activation prevents this behavior.

Finally, we have recently shown that pair bonded males show a significant increase in D1-type, but not D2-type, receptor density in the NAcc but not in other DAergic brain areas compared with controls (Aragona et al. 2003b). Given that D1-type activation prevents pair bond formation, this modification of the brain may prevent the formation of new pair bonds and therefore promote the maintenance of the already formed bond. D1- and D2-type receptor distributions and the effects of mating/social experiences on these receptors are being compared between monogamous and non-monogamous voles in an ongoing experiment in our laboratory.

Neurochemical Interactions

Complex social behaviors such as pair bonding involve many processes including, but not limited to, sensory processing, motivation, attention, memory, and locomotor outputs. Given the incredible complexity involved in pair bonding, it is not surprising to learn that this behavior is under the control of many neurochemical systems. Rather than functioning independently, these systems likely interact and share some common mechanisms in the regulation of pair bonding. Although relatively few studies have been performed, the current data suggest that this is indeed the case for AVP, OT, and DA systems.

Central administration (icv) of AVP or OT induces part-

ner preference formation in prairie voles, whereas administration of the V1a antagonist or OTA blocks this behavior induced by either neuropeptide (Cho et al. 1999). In male prairie voles, in particular, AVP infused into LS induces partner preference formation, and this behavior is blocked by coadministration of either the V1a antagonist or OTA (Liu et al. 2001b). These data suggest that access to both AVP and OT receptors in LS is necessary for AVP-induced partner preference formation. Furthermore, although DA is involved in pair bond formation in prairie voles (Aragona et al. 2003a; Gingrich et al. 2000; Wang et al. 1999), mating induces DA release in NAcc in other species of rodents that do not form pair bonds (Mermelstein and Becker 1995; Pfaus et al. 1995), and mating induces DA release in dorsal striatum similarly in both monogamous and non-monogamous voles (Curtis et al. 2003). Therefore, DA alone cannot explain pair bond formation found in monogamous voles. Instead, DA involvement in pair bonding may be due to its interactions with other neurochemcial systems, which differ between monogamous and non-monogamous voles. Indeed, NAcc was initially implicated in pair bonding because prairie voles have more OT receptors in NAcc compared with non-monogamous voles, and intra-NAcc administration of OTA blocked mating-induced partner preferences in female prairie voles (Insel and Shapiro 1992; Liu and Wang 2003; Young et al. 2001).

In a recent study, administration of OTA in NAcc blocked partner preferences induced by either OT or the D2-type agonist quinpirole, whereas the D2-type antagonist eticlopride, blocked the same behavior induced by either quinpirole or OT in female prairie voles (Liu and Wang 2003). These data indicate that concurrent activation of OT and D2-type DA receptors in NAcc is necessary for partner preference formation in female prairie voles. In the same study, as expected, a D1-type antagonist did not block partner preferences induced by OT.

Finally, the primary output area of NAcc is the ventral pallidum (Heimer et al. 1991), an area enriched with the V1a receptors (Wang et al. 1997b) and also involved in pair bonding (Lim and Young 2002). Given the large degree of interconnection between NAcc and ventral pallidum, it is possible that DA and AVP systems interact to influence pair bond formation. At present, AVP-OT manipulations in LS have been performed only in male prairie voles (Liu et al. 2001b), whereas DA-OT interactions in NAcc have been studied in female prairie voles (Liu and Wang 2003). Given the fact that no sex differences are found in AVP/OT receptor distributions (Insel et al. 1994; Wang et al. 1997b; Young et al. 1997), it would be interesting to study these neurochemical interactions in the regulation of pair bonding in both males and females.

Glucocorticoid Regulation of Pair Bonding

Up to this point, pair bonding that is induced or enhanced by mating has been the focus of this review. However, non-sexual stimuli can also influence pair bonding. For instance, female prairie voles show partner preferences and selective aggression in the absence of mating if the period of cohabitation is long enough (Bowler et al. 2002; Williams et al. 1992). This behavior makes ecological sense because female voles do not cycle and estrous is induced by the extended presence of a novel male (Richmond and Conaway 1969). Therefore, nonsexual encounters may affect mate choice and perhaps partner preference formation in females. One physiological effect of encountering a novel individual is altered levels of stress hormones, such as corticosterone (CORT[1]).

Prairie voles exhibit hypersecretion of glucocorticoid under basal conditions and attenuated end-organ responses to glucocorticoid challenge (decreased abundance and affinity of glucocorticoid receptors) and are therefore termed glucocorticoid resistant (Taymans et al. 1997). Although there are very high levels of CORT (about 10 times that of the rat), there is also less expression of adrenal steroid receptors that have lower binding affinity in prairie vole hippocampus compared with animals that have typical levels of CORT (Hastings et al. 1999). Prairie voles, as highly social animals, show remarkable behavioral and physiological responses to changes in social environment, and CORT has been used as an indicator of such responses. For example, as pups, social isolation increases ultrasonic distress calls in prairie, but not montane, voles; and there is a corresponding increase in CORT only in the prairie voles (Shapiro and Insel 1990). As adults, social isolation increases, whereas exposure to males decreases CORT levels in sexually naive female prairie voles (DeVries et al. 1995; Kim and Kirkpatrick 1996). If females are pair bonded, however, exposure to an unfamiliar male appears to elevate CORT levels (DeVries et al. 1995).

The experimental data indeed indicate that CORT is involved in pair bond formation in prairie voles. Adrenalectomized females showed partner preferences after 1 hr of cohabitation with a male in the absence of mating, whereas CORT treatment prevented this behavior, suggesting that a decrease in CORT may facilitate partner preference formation in female prairie voles (DeVries et al. 1995). Interestingly, CORT treatment on intact females even induced preferences for strangers, further demonstrating the role of CORT in social choice: Decreases in CORT lead to partner preference formation, whereas increases in CORT lead to avoidance of the partner in female prairie voles (DeVries et al. 1995). Unlike females, peripheral injections of CORT induced partner preferences in the absence of mating in males (DeVries et al. 1996). Furthermore, a brief swim stress, which increases circulating CORT, induced partner preference formation in males, and this behavior was prevented by previous adrenalectomy (DeVries et al. 1996). Conversely, the same swim stress did not induce partner preferences in intact females (DeVries et al. 1996). Central administration of corticotropin-releasing factor in males also induces partner preferences in the absence of mating (DeVries et al. 2002). Together, these results indicate that

CORT involvement in pair bonding is sexually dimorphic with increased CORT facilitating partner preference formation in males but antagonizing the same behavior in females.

Given the sexual dimorphism in CORT regulation of pair bonding, some unexpected results have been reported. For example, a similar swim stress increased hypothalamic corticotropin-releasing factor mRNA equally in male and female prairie voles (Liu et al. 2001a). In addition, liposaccarides, which increase CORT in both sexes, induced partner preferences in females but had no effects in males (Bilbo et al. 1999). It is also worth noting that the 1-hr cohabitation paradigm has been used to study CORT involvement in pair bonding, whereas the majority of studies addressing neuropeptide/neurotransmitter involvement have used different behavioral paradigms by focusing on mating-induced pair bonding. Therefore, it is difficult to speculate about potential interactions between CORT and OT/AVP/DA in pair bonding. In other rodents, there are examples demonstrating that CORT manipulations can alter levels of OT, AVP, and DA (Mahata et al. 1993), and that OT/AVP/DA are involved in stress responses and may induce CORT release (Ikemoto and Panksepp 1999; Whitnall 1993).

Potential Mechanisms of Pair Bonding

By what mechanisms may the implicated neurochemicals regulate pair bonding? Expression of a partner preference requires that subjects reliably discriminate between partner and stranger. Therefore, neurochemical manipulations that affect partner preferences may do so by altering social recognition. This hypothesis is supported by the findings that AVP in the LS is important in individual recognition, particularly in male rodents (Bluthe and Dantzer 1990; Dantzer et al. 1988; Engelmann et al. 1996), and that social amnesia (inability to recognize individual conspecifics) displayed by OT knockout mice is reversed by OT replacement (Ferguson et al. 2002). Neurochemicals may also act on the formation of pair bonds. Administration of the D2-type antagonist before mating blocks partner preferences. However, administration of the same drug after mating (just before the partner preference test) did not block mating-induced partner preferences in female prairie voles, suggesting that D2-type activation is involved in the formation, but not the expression, of pair bonding (Wang et al. 1999).

By what mechanism might neurochemicals act on the formation of partner preferences? It has been suggested that OT/AVP/DA/CORT systems are involved in altering motivation and that activation of these systems may be involved in reward-related behavior (Insel and Young 2001; Goeders 2002; Kovacs et al. 1998; Wise and Rompre 1989). Forebrain areas such as NAcc and ventral pallidum, which are associated with reward learning, are involved in the neurochemical regulation of pair bonding, indicating that there may be a significant reward component to partner preference formation. Therefore, one working hypothesis concerning how pair bonds are formed is that when two individuals of the opposite sex meet, they are initially neutral to one another. If the appropriate social interaction leading to a rewarding event occurs, then the individual is subsequently preferred to other potential mates. This preference is stable over time in prairie voles and therefore results in a monogamous life strategy.

Future Directions

This review describes our current knowledge of the neurochemical regulation of pair bonding based on studies using the prairie vole model system. Future work will continue to define neural circuits, neurochemical interactions, and the cellular and molecular mechanisms underlying pair bonding. In addition, there is growing momentum for the use of prairie voles in several new lines of research, which are very interesting but difficult to perform with traditional laboratory rodents.

Unlike laboratory rodents that have been inbred for many generations, the typical prairie vole colony is closer to wild populations, and it is a common practice to outbreed colonies to maintain genetic variation (Hammock and Young 2002). As a result, there is much more individual variability in the behavior of voles compared with mice or rats (Ranson 2003). As briefly noted above, prairie voles have an insert in the promoter region of their AVP V1a receptor gene of approximately 400 bp, and this insert is implicated in social behavior (Young et al. 1999). Recently, it has been shown that the length of this insert is highly variable among individual prairie voles and is related to the eventual distribution patterns of the V1a receptors in the brain (Hammock and Young 2002). It is possible that the distribution patterns of this receptor regulate social behaviors that are under selection pressures in nature. For this reason, studies of this promoter region in prairie voles can serve as a model to study the evolution of complex social behavior.

The prairie vole has also been successfully used to examine adult neurogenesis. Although recent studies have revealed adult neuron proliferation in a variety of mammalian species and have identified some of the factors that influence the rates of adult neurogenesis, the functional significance of these new cells remains unknown. In adult voles, newly proliferated cells are found in selected brain regions, and environmental factors such as mating, social isolation, and seasonal changes, as well as endogenous factors such as gonadal steroid hormones and stress hormones, significantly influence the rate of cell proliferation and survival (Fowler et al. 2002; Galea and McEwen 1999; Ormerod and Galea 2001; Smith et al. 2001). In prairie voles, in particular, manipulation of the social environment not only influences their social behaviors but also alters neurogenesis in brain regions involved in the regulation of social behavior (e.g., amygdala; Fowler et al. 2002). Therefore, the prairie vole may provide an opportunity to investigate the functional significance of newly proliferated cells and, specifi-

cally, whether these new cells are important for pair bonding.

Conclusion

In summary, prairie voles allow us to ask questions about the neurobiology of complex social behaviors generally difficult to address using traditional laboratory rodents. Detailed analysis of the neural processing of social information may be of tremendous benefit to understanding human disorders of a social nature, such as autism, social anxiety, and schizophrenia. The neurobiology of such disorders is inherently difficult to study due, in part, to the lack of an appropriate animal model. Using prairie voles to study social behavior has been very fruitful, and it is hoped that continued examination of the neural mechanisms of pair bonding will aid in our understanding of social behavior and related disorders.

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