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Neuropeptidergic regulation of affiliative behavior and social bonding in animals

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Abstract

Social relationships are essential for maintaining human mental health, yet little is known about the brain mechanisms involved in the development and maintenance of social bonds. Animal models are powerful tools for investigating the neurobiological mechanisms regulating the cognitive processes leading to the development of social relationships and for potentially extending our understanding of the human condition. In this review, we discuss the roles of the neuropeptides oxytocin and vasopressin in the regulation of social bonding as well as related social behaviors which culminate in the formation of social relationships in animal models. The formation of social bonds is a hierarchical process involving social motivation and approach, the processing of social stimuli and formation of social memories, and the social attachment itself. Oxytocin and vasopressin have been implicated in each of these processes. Specifically, these peptides facilitate social affiliation and parental nurturing behavior, are essential for social recognition in rodents, and are involved in the formation of selective mother–infant bonds in sheep and pair bonds in monogamous voles. The convergence of evidence from these animal studies makes oxytocin and vasopressin attractive candidates for the neural modulation of human social relationships as well as potential therapeutic targets for the treatment of psychiatric disorders associated with disruptions in social behavior, including autism.

Keywords: Vasopressin receptor; Oxytocin receptor; Social recognition; Social behavior; Pair bond; Autism; Neuropeptides

Introduction

Healthy social relationships are essential for proper mental health and many psychiatric disorders are associated with disruptions in social motivation and the ability to maintain social relationships (Bowlby, 1977; House et al., 1988; Kiecolt-Glaser and Newton, 2001; Monroe et al., 1986). Relationships among spouses, family, and friends are universally important across all human societies, yet little is known about the neurobiological mechanisms underlying the development and maintenance of such human relationships. Aside from a handful of postmortem studies and more recent functional imaging approaches, the neurobiology of human social behavior has been difficult to study. Fortunately, research using animal models has begun to provide insights into the social brain and the regulation of social relationships. Although the research in this field is far from complete, these animal models can serve to complement existing data on normal human social behavior and guide investigations of the neurobiology of pathological sociality, such as in autism spectrum disorders (see Bartz and Hollander, 2006).

The formation and maintenance of social relationships are a complex process that involves several levels of information processing in the brain. For both ease and clarity, animal models of social behavior have generally focused on a single level of processing at a time. Therefore, we have developed a simplified conceptual framework as a useful heuristic tool for understanding the neurobiology of social bonds, and we will follow that framework in this review. First, the organism must be motivated to approach and engage another individual. Next, the

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animal must be able to identify the individual based on social cues through the formation of social memories. Finally, given the appropriate conditions, a bond can form, leading to preferential interaction with that individual. Each of these conceptual levels engages different brain regions and neural circuits. Thus, neuropathology can occur at any level of this framework, with the resulting phenotype being a global impairment in the development of social relationships. This chapter will discuss the animal models developed for each of the three levels with a focus on the neuropeptides oxytocin and vasopressin as a preface to the following review, which will discuss translational implications relevant to these basic neuroscience discoveries (Bartz and Hollander, 2006).

Background

The neurohypophyseal hormones oxytocin and vasopressin play central roles in the regulation of affiliative behavior and social bonding in animals. Oxytocin is best known for its reproductive role in the peripheral circulation, particularly in contraction of the uterus during labor and ejection of milk during lactation (Burbach et al., 2006). Oxytocin is synthesized in magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus (PVN and SON, respectively), which project to the neurohypophysis, or posterior pituitary, and release the peptide into the peripheral circulation. Oxytocin is also produced within the parvocellular neurons of the PVN, which project to limbic sites such as the hippocampus, amygdala, striatum, hypothalamus, nucleus accumbens, and to mid- and hindbrain nuclei such as the locus coeruleus and nucleus of the tractus solitarius, as well as the spinal cord (Sofroniew, 1983). Oxytocin released within the brain itself is thought to regulate behavior by acting as a neurotransmitter/neuromodulator.

Vasopressin is a closely related peptide, also nine amino acids in length, best known for its actions as anti-diuretic hormone at V2 receptors in the kidney. It is thought that the genes for oxytocin and vasopressin emerged from the duplication of a single ancestral nonapeptide gene early in vertebrate evolution; they are highly conserved in structure and function across taxa. Like oxytocin, vasopressin is synthesized in magnocellular PVN and SON neurons and released from the posterior pituitary into the peripheral circulation. Vasopressin is also synthesized within parvocellular neurons in the PVN and suprachiasmatic nucleus as well as in extrahypothalamic neurons in the bed nucleus of the stria terminalis and medial amygdala (de Vries and Miller, 1998; De Vries and Panzica, 2006). These extrahypothalamic sources of vasopressin are androgen dependent and are the likely source of sexually dimorphic projections within the brain (de Vries and Miller, 1998).

Centrally released oxytocin and vasopressin have been implicated in the regulation of a wide range of social behaviors, some of which will be discussed in detail below. Oxytocin facilitates social motivation and approach behavior, including maternal nurturing behaviors (Burbach et al., 2006). Vasopressin regulates several male-typical social behaviors, including scent marking, aggression, and paternal care (Boyd et al., 1992; Delville et al., 1998; Ferris et al., 1990; Goodson and Bass, 2001; Wang et al., 1994). Both oxytocin and vasopressin are important for the formation or expression of social memories required for the discrimination of familiar individuals (Bielsky and Young, 2004). Both peptides are also involved in pair bond formation in monogamous prairie voles (Young and Wang, 2004). Thus, both oxytocin and vasopressin are heavily involved at each of the conceptual levels leading to social bonding: The initial approach and affiliation, the recognition of social cues required for individual recognition, and finally the formation of the bond itself. Each of these processes will be discussed separately below.

Social approach and motivation

The neurobiology of social approach and motivation can be studied by measuring the latency time to approach another individual and the amount of time spent in social contact. Here we discuss the role of oxytocin and vasopressin in three general animal models of social approach and motivation: parental behavior, infant–mother interactions, and adult affiliation. At this conceptual level, social motivation is primarily non-selective in nature. For example, maternal female rodents direct maternal nurturing to any pup, regardless as to whether they are their own.

Mother–infant care can be studied by examining the behavioral components of maternal care, which includes nest building, licking and grooming pups, and crouching over pups. Maternal nurturing behavior develops coincident with labor and parturition. Virgin female rats initially find pups aversive and will actively avoid them (reviewed in Fleming and Anderson, 1987). After parturition, rats find pups rewarding and can actually be trained to bar press to gain access to pups (Lee et al., 1999). Oxytocin originating from the PVN or SON may act on oxytocin receptors throughout the brain to promote maternal responsiveness. Lesions of the PVN result in a near complete loss of the brain oxytocinergic system (De Vries and Buijs, 1983), and a delay in the onset of maternal behavior in naïve rats (Insel and Harbaugh, 1989). Oxytocin injected intracerebroventricularly (i.c.v.) into virgin female rats induces maternal behavior (Pedersen et al., 1982). Similarly, i.c.v. oxytocin injected into both virgin and pregnant wild house mice also increases maternal behavior towards pups (McCarthy, 1990). In contrast, oxytocin receptor antagonists delivered to the ventricles delay the onset of maternal behavior in hormone-primed females (Fahrbach et al., 1985; van Leengoed et al., 1987). Finally, oxytocin receptor antagonists injected directly into the medial preoptic area (MPOA) and ventral tegmental area (VTA) inhibit maternal behavior (Pedersen et al., 1994).

Oxytocin and oxytocin receptor (OTR) levels in the brain are regulated by estrogen and progesterone followed by progesterone withdrawal (Amico et al., 1997; Bale et al., 1995). During pregnancy, when estrogen levels rise, OTR expression increases in the hypothalamus and MPOA (Young et al., 1997a). Treatment with estrogen also results in increased levels of OTR via estrogen receptor alpha activation (Breton and Zingg,

1997; Young et al., 1998). Thus, it is hypothesized that the hormones of pregnancy prime the brain's ability to respond to oxytocin released during parturition. In addition, OTR density within brain regions such as the central nucleus of the amygdala and bed nucleus of the stria terminalis (BnST) is correlated with individual variation maternal care (Francis et al., 2000, 2002).

Oxytocin significantly decreases infanticidal behavior in wild house mice (McCarthy, 1990). Infanticide in this instance is postulated as an adaptive behavior to maximize resources for when the female has her own offspring. In contrast, some strains of laboratory mice show spontaneous maternal behavior when exposed to pups from other mice. The role of oxytocin in mouse maternal behavior was questioned when it was reported that the oxytocin knockout mice display grossly intact maternal behavior (Nishimori et al., 1996; Young et al., 1996b). However, later studies reported that in semi-naturalistic conditions, oxytocin knockout mice did in fact display impaired maternal behavior (Ragnauth et al., 2005). More recent studies of OTR knockout mice reveal pervasive social deficits including spontaneous and parturient maternal nurturing of pups (Takayanagi et al., 2005). In addition, the *peg3* (paternally expressed gene 3) knockout mouse has reduced numbers of oxytocin neurons in the PVN and has a profound deficit in maternal behavior with a complete absence of nest building, pup retrieval, and crouching behaviors (Li et al., 1999). In wild-type animals, PEG3 is present in brain areas involved in maternal behavior such as the MPOA, BnST, PVN and medial amygdala (Li et al., 1999). These data suggest that PEG3 activity may be upstream of oxytocin and other genes involved in the neural circuitry of maternal behavior, and PEG3 may contribute to the developmental organization of the behavior.

Prairie voles (*Microtus ochrogaster*) are monogamous rodents that display biparental care of the young and pair bond formation between adult mates. Approximately half of adult female prairie voles are spontaneously maternal (i.e., as virgins), but unlike mice, juvenile prairie voles (21 days of age) display spontaneous alloparental care of pups (Solomon, 1991; Wang and Novak, 1994). Oxytocin is critical for the expression of spontaneous maternal behavior in both adult female and juvenile prairie voles (Olazabal and Young, 2006a, 2006b). In juvenile prairie voles, the density of OTR binding in the nucleus accumbens (NAcc) is positively correlated with the time spent crouching over pups, an aspect of maternal behavior ($r=0.69$, Pearson's correlation) (Fig. 1C) (Olazabal and Young, 2006a). In adult females, microinjections of OTR antagonist directly into NAcc block spontaneous maternal behavior (Olazabal and Young, 2006b). Interestingly, non-monogamous rodents such as rats, mice, and meadow voles have very little OTR binding in NAcc at baseline (Olazabal and Young, 2006a). The NAcc is a key component in natural reward and reinforcement circuits of the brain, suggesting that maternal behavior may have a strong motivational and reinforcement component.

Unlike non-monogamous rodent species, prairie vole males display high levels of paternal care. Initial studies suggested that vasopressin stimulates paternal behavior in male prairie voles when infused directly into the lateral septum, as assessed by increased time spent crouching over and licking/grooming pups (Wang et al., 1994). Likewise, paternal behavior was blocked by injection of vasopressin V1a receptor-selective antagonist (Wang et al., 1994). V1aR binding patterns were associated with individual variation in the degree of paternal care delivered in a facultatively paternal species, the meadow vole, as well as within monogamous prairie voles (Hammock et al., 2005; Parker et al., 2001).

Infant–mother interactions also require intact social approach and motivation circuits. There are fewer studies on the desire of the infant to seek the mother, and most involve knockout mice. A preliminary study using oxytocin knockout mice revealed an increased latency of the pup to crawl to the mother, when separated from the mother, and decreased separation distress, as evidenced by fewer ultrasonic vocalizations when separated from the mother (Young et al., 1997c; Fusaro and Young, unpublished data). Similarly, mu-opioid receptor knockout pups show fewer ultrasonic vocalizations when separated from the mother and decreased preference for the mother's scent (Moles et al., 2004). This suggests that both oxytocin and opioid systems are involved in the motivation of an infant to seek social contact with its mother, perhaps also acting through natural reward circuits in the brain.

Affiliation between adults can also be viewed as a measure of social approach and motivation. Chronic infusion of oxytocin directly into the brain increases social contact time between adult rats (Witt et al., 1992). Similarly, affiliation between monogamous female Mongolian gerbils is increased by subcutaneous injections of oxytocin (Razzoli et al., 2003). In non-human primates, two macaque species with natural species differences in affiliative behavior exist: the naturally gregarious and affiliative bonnet monkey has increased levels of CSF

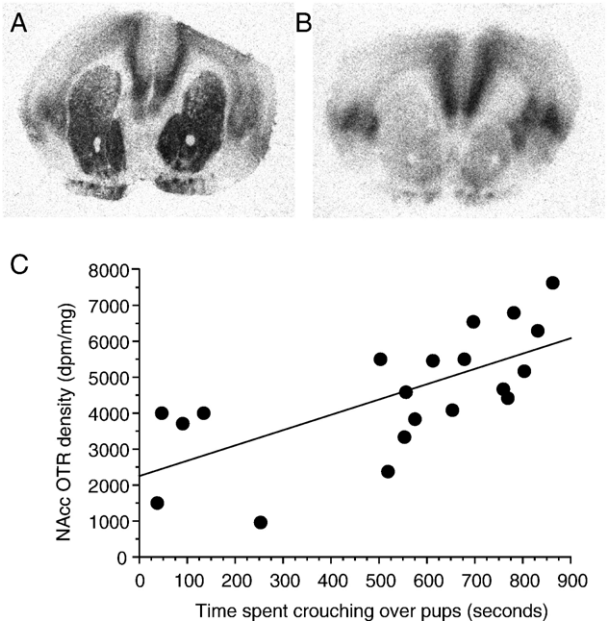

Fig. 1. Social approach/motivation. Individual variation in OTR binding in NAcc in juvenile prairie voles. (A) Individual with high OTR binding in NAcc. (B) Individual with low OTR binding in NAcc. (C) NAcc OTR binding density is positively correlated with the amount of time spent crouching over pups ($r=0.69$, Pearson's correlation) (Olazabal and Young, 2006a, with permission from Elsevier).

oxytocin compared to the relatively asocial pigtail macaque (Rosenblum et al., 2002).

Among voles, vasopressin infusions increase the amount of social interaction in the highly social prairie vole, but not in the asocial montane vole (Young et al., 1999). This difference in behavioral response to vasopressin appears to be due to species differences in vasopressin receptor expression pattern in the brain, since transgenic mice engineered to express the vasopressin receptor in a "prairie vole-like" brain pattern (see below) also show increased social contact after vasopressin injections (Young et al., 1999). Increasing the number of vasopressin receptors in the brain using viral vector gene transfer increases social contact time between adult rats (Landgraf et al., 2003), and between adult male and juvenile prairie voles (Pitkow et al., 2001). Taken together, these data suggest that both oxytocin and vasopressin seem to be involved in the social motivation to seek contact and approach another individual, the first step to forming a social bond.

Social recognition

The recognition of a familiar individual and the formation of a social memory of that individual is the next major step to forming a social bond. The neurobiology of social recognition and memory in rodent models can be studied in the laboratory by measuring the duration of social investigation during subsequent exposures to the same individual. This behavioral assay is based upon the phenomenon that rodents investigate novel items (or individuals) longer than familiar items (or individuals). Thus, if a rodent recognizes a familiar individual, it will spend significantly less time investigating that individual with subsequent exposures (Winslow, 2003). Since social recognition involves the processing of social cues, social recognition may serve as a more general model for the neural processing of social information, which may be of particular importance for disorders such as autism.

In humans, social recognition is primarily visual in nature, and selective lesions in a single brain region, the fusiform gyrus, can abolish the ability to recognize faces (known as prosopagnosia) (Barton et al., 2002). In rodents, olfaction appears to be more important for social recognition, and of particular importance is the vomeronasal organ system which projects to the accessory olfactory bulb to detect pheromones. In the visual system, projections synapse within the fusiform gyrus and superior temporal sulcus. In the olfactory system, the projections are instead routed through the piriform cortex and amygdala. The amygdala has been implicated in the processing of social emotions and memory (Adolphs, 2003). In rodents, we have been able to study the flow of olfactory information from the olfactory bulb to more downstream sites such as the amygdala, lateral septum, and cortex and their respective involvements in social recognition.

Early pharmacological studies beginning nearly 20 years ago first demonstrate a modulatory effect of oxytocin on memory and social recognition in rodents (Dantzer et al., 1987; Popik et al., 1992a,b). Transgenic mice that lack the oxytocin gene, and therefore do not produce the oxytocin peptide, are unable to recognize familiar individuals despite repeated exposures (Fig. 2A) (Choleris et al., 2003; Ferguson et al., 2000). This is not due to a generalized deficit in olfaction or learning and memory, as these mice can habituate to a non-social scent and perform normally on spatial memory tasks (Choleris et al., 2003; Ferguson et al., 2000). The profound social deficits of the oxytocin knockout mice can be temporarily restored by a single injection of oxytocin into the lateral ventricles of the brain just prior to, but not after the initial encounter with a conspecific (Ferguson et al., 2001).

In order to investigate the neural circuits of social recognition modulated by oxytocin in mice, Fos activation was examined in the olfactory processing circuit during a social encounter, comparing mutant to wild-type mice. Wild-type mice showed brain activation in the olfactory bulbs, piriform cortex, and medial amygdala during the 90-s social encounter. In contrast, oxytocin knockout mice only showed neural activity in the olfactory bulbs and piriform cortex, but not in the medial amygdala (Fig. 2A) or in downstream targets of the medial amygdala, such as the bed nucleus of the stria terminalis and the MPOA (Ferguson et al., 2001). Intriguingly, the knockout mice showed a massive activation of other brain regions, such as the cortex and hippocampus, that was absent in the wild-type mice (Ferguson et al., 2001). These results led to the discovery that microinjections of oxytocin specifically into the amygdala, but not the olfactory bulb, could rescue social recognition deficits in the oxtyocin knockout mice (Ferguson et al., 2001). Thus, in mice, oxytocin acts at the amygdala during a social encounter for the normal processing of social information required for intact social recognition. In the absence of oxytocin, social information appears to be processed by alternative neural circuits, such as cortical and hippocampal regions, but social recognition fails to develop.

Alternative neural processing also appears to occur in autistic patients during processing of social cues, and this is evident from several neuroimaging studies. When viewing images of human faces, autistic patients show decreased amygdala and fusiform gyrus activation, and increased cortical activation compared to normal subjects (Critchley et al., 2000; Pierce et al., 2001; Schultz et al., 2000). When asked to interpret emotions based by judging expression from another person's eyes, autistics failed to show amygdala activation seen in normal subjects (Baron-Cohen et al., 1999). This is strikingly reminiscent of the findings reported in the oxytocin knockout mice, which also showed absent amygdalar activation and recruitment of cortical areas (Ferguson et al., 2001). One possibility for decreased amygdala activation is that autistic patients prefer to avoid looking directly at faces ("gaze aversion"), and in fact one study showed that autistics may show exaggerated amygdala activity when looking directly at faces (Dalton et al., 2005). In sum, whether there is diminished or heightened amygdala activity, the evidence points to global dysregulation of the neural circuits for processing of social information.

Oxytocin also acts in other brain areas in addition to the amygdala to control social recognition. Oxytocin infused into the olfactory bulbs of rats prolongs the length of time the test rat

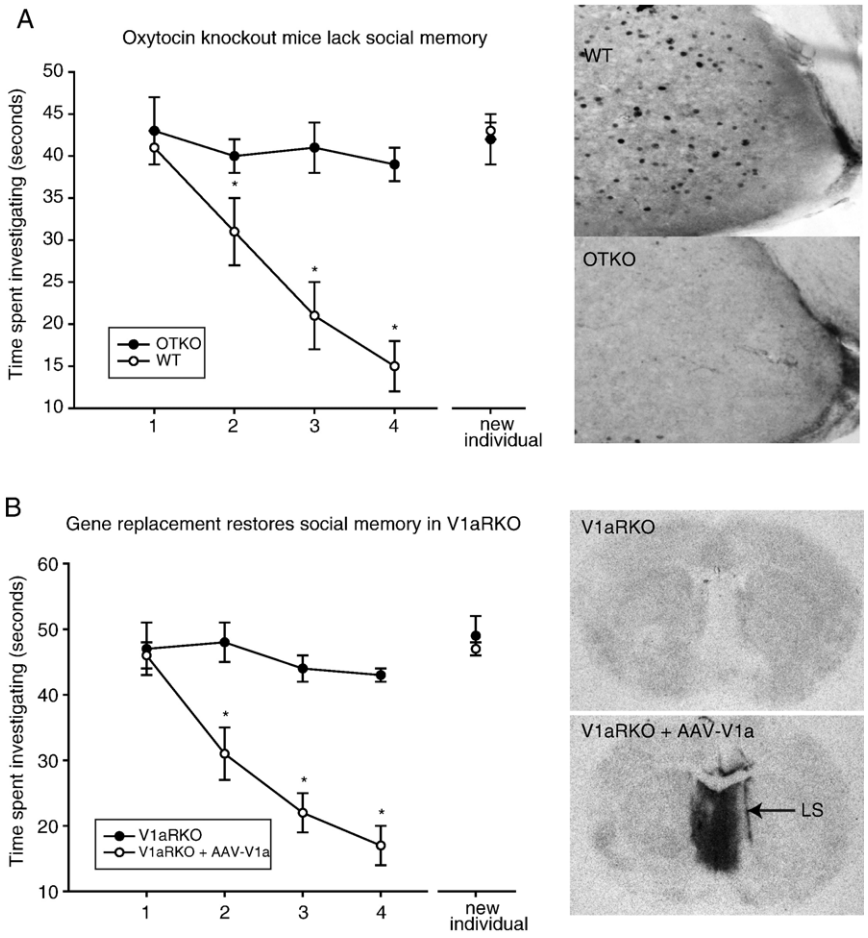

Fig. 2. Social recognition. (A) Oxytocin knockout (OTKO) mice spend an equivalent amount of time investigating another individual despite repeated exposures. Wild-type mice habituate with each successive exposure but will reinvestigate a novel individual. Fos activation is induced in the medial amygdala after a social exposure in wild-type mice, but not in OTKO mice (Ferguson et al., 2001). (B) V1aR knockout mice show a similar social amnesia. Deficits in social memory can be rescued by replacing V1aR expression using viral vector gene transfer (AAV-V1a) in the lateral septum (Bielsky et al., 2005, with permission).

remembers another individual, and this effect is modulated by norepinephrine (Dluzen et al., 1998). Oxytocin treatment results in increased norepinephrine release as measured by microdialysis of the bulb (Dluzen et al., 2000). Lesions of norepinephrine cells using 6-hydroxydopamine in the bulb prevent oxytocin from extending the duration of social recognition (Dluzen et al., 1998), while stimulation of alpha-2 noradrenergic receptors with clonidine or blockade of norepinephrine reuptake by nisoxetine increase the duration of social memory (Dluzen et al., 2000). Oxytocin injections into the lateral septum and MPOA of male rats can prolong social recognition (Popik and van Ree, 1991; Popik et al., 1992b).

Vasopressin also plays a role in rodent social recognition. Vasopressin itself was first implicated in the 1960s in avoidance learning and memory (van Wimersma Greidanus et al., 1983). The Brattelboro rat, a naturally occurring vasopressin-deficient mutant, displays a total disruption of social recognition (van Wimersma Greidanus, 1982). Social memory in the Brattelboro rat can be restored by infusing vasopressin into the lateral septum (Engelmann and Landgraf, 1994). Peripheral, intracerebroventricular (i.c.v.) and intraseptal administration of the vasopressin V1a receptor (V1aR) antagonist have all been shown to block social recognition in normal rats (Dantzer et al., 1987). Likewise, intraseptal injection of V1aR antisense oligonucleotides also results in impaired social memory (Dantzer et al., 1987; Everts and Koolhaas, 1999; Landgraf et al., 1995). Furthermore, viral vector-mediated over-expression of V1aR specifically in the lateral septum can facilitate social recognition in normal rats by prolonging the duration of social memory (Landgraf et al., 2003).

Investigation of vasopressin in mice also points to a critical role in social memory. Transgenic male mice with a null mutation in the gene encoding the V1aR, *avpr1a*, lack the ability to recognize a familiar conspecific, despite repeated exposures (Fig. 2B) (Bielsky et al., 2004). This social deficit is not a result of a general olfactory deficiency, given the ability of these mice to habituate to a non-social stimulus. Furthermore, spatial learning and memory and sensorimotor processing are also normal in the V1aR knockout mice, suggesting that the deficit is specific for the learning and/or recall of social cues (Bielsky et al., 2004). Replacement of V1aR directly into the lateral septum in *avpr1a* knockout mice using viral vector gene

transfer completely restores social recognition in these animals (Fig. 2B) (Bielsky et al., 2005). Another vasopressin receptor subtype, the V1b receptor (V1bR), has recently been localized to the brain, and the *V1bR* knockout mouse shows a modest disruption in social memory, suggesting that the V1bR subtype may also play a role in social memory (Hernando et al., 2001; Wersinger et al., 2002).

Although social recognition is a complex behavior, it is clear that both oxytocin and vasopressin systems are involved in the ability to process social information and likely work in concert to regulate the ability to recognize familiar conspecifics and form long-term memories of them.

Social bond formation

Social bonding is a complex social behavior that requires the integration of many cognitive processes including social approach, motivation, and memory formation. Once an individual is motivated to approach another individual and forms a memory of that individual, then the stage is set for the formation of a social relationship. Animal models of attachment behavior are different from the general, non-selective maternal or affiliation behaviors described earlier; attachment bonds between individuals are both selective (i.e., with rejection of strangers) and enduring. There are two excellent animal models of social bonding. The first examines the neural substrates of the highly selective bond between the sheep mother and her lamb, while the second examines pair bond formation between two adult mates in the monogamous prairie vole.

Sheep have become a very useful model of attachment and bonding, because like rats, they also show strong maternal behavior after parturition. However, in contrast to rats and mice, the ewe shows highly selective maternal behavior only with her own lamb. Many of the same molecular correlates for maternal behavior and social recognition in rats and mice have emerged in this model of mother–infant attachment in sheep. Like rats, the changes in hormones during pregnancy and the events of labor and delivery are required for the onset of maternal behavior in ewes. Hormone priming with estradiol and progesterone followed by vagino-cervical stimulation induces maternal behavior in virgin ewes (Keverne et al., 1983). Vagino-cervical stimulation results in increased oxytocin release measured in cerebrospinal fluid and in the brain using microdialysis (Kendrick et al., 1986, 1988a,b). As sheep farmers have known for centuries, vagino-cervical stimulation can induce acceptance of an unfamiliar lamb even after the mother has bonded with her own lamb (Kendrick et al., 1991). Epidural anesthesia blocks these effects but can be overcome by exogenous intracerebroventricular oxytocin, suggesting that the ascending sensory input from the vagina is important for inducing maternal acceptance, perhaps via increasing the release of oxytocin in the brain (Levy et al., 1992). In fact, oxytocin injection alone can induce acceptance of an unfamiliar lamb even in a non-pregnant ewe (Kendrick et al., 1987).

Oxytocin is clearly involved in the switch to maternal behavior in sheep, but what controls the generation of a persistent, selective attachment between the ewe and her lamb? Ewes become maternal immediately after delivery; however, within a few hours, they become selectively maternal and will only accept their own lamb to nurse. This behavior is thought to be mediated in part by reorganization of the olfactory bulb (Kendrick et al., 1992). Vagino-cervical stimulation results in changes in various neurotransmitters in the sheep olfactory bulb, including oxytocin, noradrenaline, acetylcholine, glutamate and GABA levels which all rise (Keverne et al., 1993; Levy et al., 1993). Oxytocin appears to modulate the release of the other neurotransmitters, suggesting that the release of oxytocin organizes the changes in the olfactory bulb after parturition or vagino-cervical stimulation (Levy et al., 1995). The cells in the olfactory bulb become very highly tuned to detect lamb odors and specifically to discriminate among lambs. The number of output neurons in the olfactory bulb, or mitral cells, increases after parturition together with increased cholinergic and noradrenergic neurotransmitter release (Kendrick et al., 1992). A subset of these mitral cells respond strongly and selectively to the specific lamb's odors and show increased glutamate and GABA signaling just in response to this lamb (Kendrick et al., 1992). The selective increase in glutamate signaling is mediated by nitric oxide acting as a retrograde messenger within the olfactory bulb (Kendrick et al., 1997).

Thus, in sheep, it is hypothesized that vagino-cervical sensory information ascends through the spinal cord and activates the PVN, which has been primed with the hormones of pregnancy to rapidly release large amounts of oxytocin into the posterior pituitary and throughout the brain. Several OTR-expressing areas (MPOA, VTA, BNST, medial amygdala and olfactory bulb) respond to the increased oxytocin release and facilitate maternal behavior in downstream neural circuits. Oxytocin release into the olfactory bulb during the first few hours after delivery allows the reorganization of the olfactory bulb (coordinating norepinephrine, glutamate and GABA signaling) such that the specific odor of the lamb is learned. These changes result in a long-lasting memory of, and selective attachment to, the lamb. The key to understanding social bond formation in the sheep model will be to link this process of selective olfactory learning to the motivation for maternal care.

As oxytocin has been implicated in maternal behavior in rats and sheep, it seems plausible that oxytocin, as well as the closely related vasopressin, might also be involved in pair bond formation between adult mates. Since mating facilitates pair bond formation, and vagino-cervical stimulation releases oxytocin in sheep, oxytocin could be similarly released during sexual intercourse in prairie voles, and thus act in the same way to cement adult–adult pair bonds. Indeed, oxytocin infused i.c.v. into both male and female prairie voles facilitates pair bond formation in the absence of mating (Cho et al., 1999; Williams et al., 1992). Similarly, vasopressin infused i.c.v. facilitates pair bond formation in both male and female prairie voles in the absence of mating (Cho et al., 1999; Winslow et al., 1993). Likewise, pair bond formation can be blocked by the i.c.v. infusion of oxytocin receptor antagonists or V1aR antagonists despite extended mating bouts (Cho et al., 1999; Williams et al., 1994; Winslow et al., 1993).

Comparison of prairie voles with closely related species suggests that differences in the distribution of oxytocin and vasopressin receptor may cause differences in social behavior. The prairie vole animal model of social attachment is complemented by the natural comparison of two other vole species, the non-monogamous montane and meadow voles (*Microtus montanus* and *Microtus pennsylvanicus*), in addition to the monogamous pine vole (*Microtus pinetorum*) (reviewed in Young and Wang, 2004). Montane voles are solitary, do not exhibit social bonding, and often abandon their young after just 2 weeks of care. All four vole species have a similar distribution of oxytocin and vasopressin projections in the brain (Wang et al., 1996), but the respective receptors, OTR and V1aR, are distributed differently between monogamous prairie and pine voles compared to promiscuous montane and meadow voles (Insel and Shapiro, 1992; Insel et al., 1994). Thus, the release of oxytocin or vasopressin would stimulate different neural circuits in monogamous versus promiscuous species, depending on which brain circuits express OTR and V1aR (Young and Wang, 2004).

Prairie voles have elevated levels of OTR and V1aR in brain regions implicated in reward and reinforcement such as the NAcc and ventral pallidum (Lim et al., 2004b) (Figs. 3A and B). In contrast, promiscuous montane and meadow voles have low levels of receptors in these regions. Microinjection of OTR antagonist into the NAcc blocks pair bonding in female prairie voles, while microinjection of V1aR antagonist into the ventral pallidum blocks pair bonding in male prairie voles (Lim and Young, 2004; Young et al., 2001) (Figs. 3A and B). Furthermore, artificial elevation of V1aR in the ventral pallidum of the promiscuous meadow vole was found to induce partner preference in this species (Lim et al., 2004c) (Fig. 3D). Thus, it

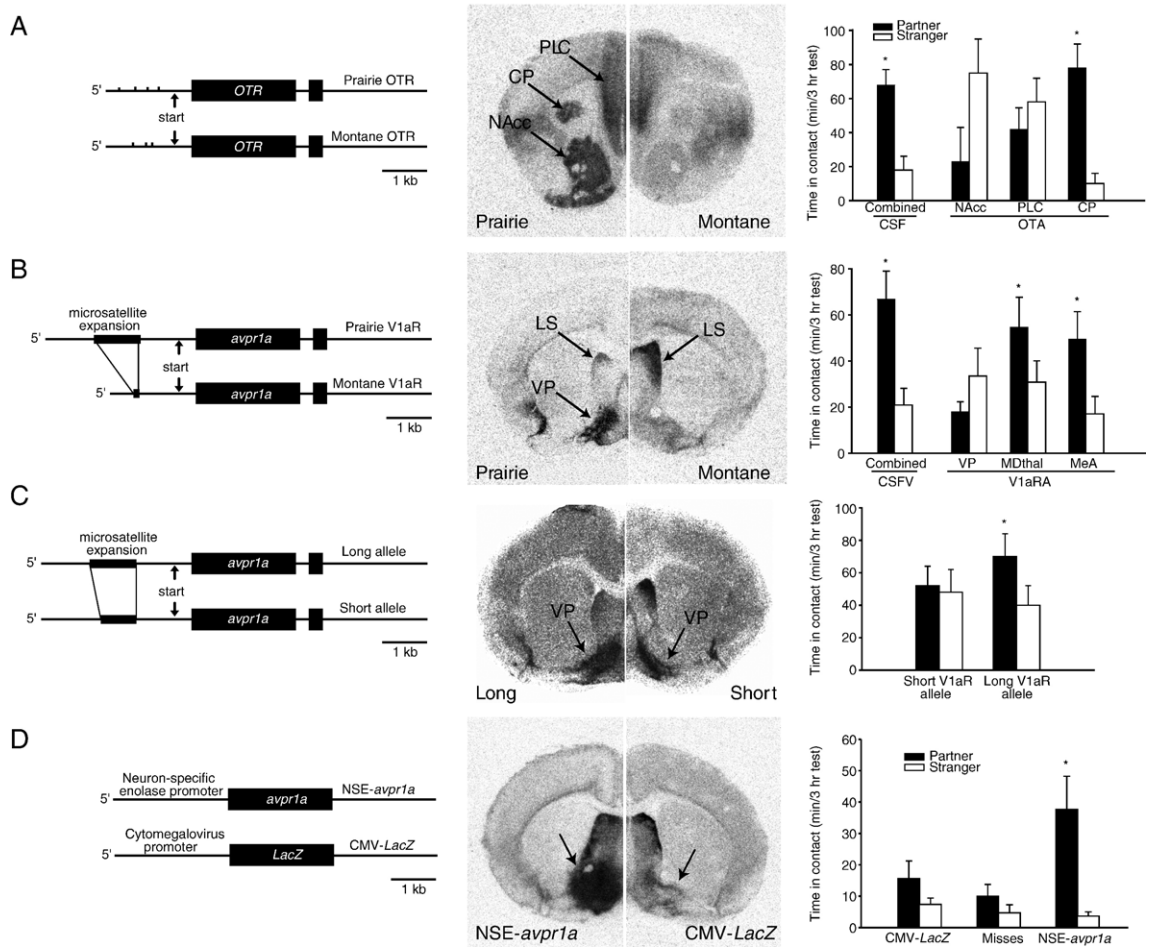

Fig. 3. Social bonding. (A) The OTR gene differs only at a few sites in the 5′ flanking region between vole species, but there are large species differences in the OTR binding pattern in the brain. OTR antagonist administration into specific regions of the brain reveal that blockade of the NAcc, but not CP, block partner preference behavior (Young et al., 2001). NAcc = nucleus accumbens; PLC = prelimbic cortex; CP = caudate-putamen. (B) The V1aR gene sequence demonstrates large species differences in 5′ flanking region, in which prairie voles have a microsatellite expansion that is very small in montane voles. V1aR binding patterns are also dramatically different between monogamous and promiscuous voles. V1aR antagonist injections into the VP block partner preference formation (Lim and Young, 2004). VP = ventral pallidum; MDthal = medial dorsal thalamus; MeA = medial amygdala. (C) Length of the microsatellite expansion varies among individual prairie voles, as does V1aR binding density within certain brain regions. Animals selectively bred to have homozygous short versus long V1aR microsatellite alleles show predictable individual variation in partner preference behavior (Hammock and Young, 2005). (D) Non-monogamous meadow voles typically exhibit low V1aR binding in VP. Artificial elevation of V1aR in VP using viral vector gene transfer (NSE-avpr1a) causes these animals to form partner preferences. Animals injected with a control virus (CMV-LacZ) did not form partner preferences (Lim et al., 2004c). Note that in all panels, the length of the microsatellite is not to scale.

appears that the specific pattern of OTR and V1aR in particular brain regions is responsible for pair bond formation in monogamous voles.

What could oxytocin and vasopressin be doing in these reward regions of the brain? We have hypothesized that the neural mechanisms of social bond formation engage the same circuitry involved in the actions of drugs of abuse. Pair bond formation in prairie voles is in fact dependent on dopaminergic neurotransmission in the nucleus accumbens (Aragona et al., 2003, 2006; Gingrich et al., 2000). A direct test of this hypothesis was performed recently. Artificial elevation of V1aR in a reward region previously lacking V1aR in the promiscuous meadow vole was found to induce partner preference formation in this species, and this behavioral switch was dependent on dopamine neurotransmission (Lim et al., 2004c). Thus, the formation of social preferences in voles is likely due to the interaction of V1aR and dopamine receptors in reward regions of the brain. This phenomenon is strikingly analogous to the formation of conditioned place preferences in the drug literature, which also depends on dopamine neurotransmission in the mesolimbic reward pathway. However, with the formation of conditioned *social* preferences, molecules involved in social processing such as oxytocin and vasopressin can now modulate reward pathways already in place.

Experimental evidence in humans supports the finding in voles that reward circuits may be involved in the neurobiology of social attachment. Two fMRI studies have examined brain activation in people while they are viewing photographs of a person the subject reported being deeply in love with. These authors observed brain activation in regions that were remarkably similar to those seen in other studies after consumption of cocaine, including dopamine reward circuits (Aron et al., 2005; Bartels and Zeki, 2000; Breiter et al., 1997; Fisher et al., 2005). Another fMRI experiment found that even simply viewing beautiful faces has reward value and activates the NAcc (Aharon et al., 2001), demonstrating that positive salient social stimuli, in this case, visual, can activate reward areas. More recent fMRI studies found that mothers viewing videos of their own infants showed significant brain activation in the prefrontal cortex (also involved in reward) compared to controls (Bartels and Zeki, 2004; Ranote et al., 2004).

What could explain the species differences in receptor distribution and thereby possibly differences in social behavior? Genetic sequencing of the prairie and montane vole OTR gene reveals a few species differences between promoter sequences (located in the 5′ flanking region of the gene) that could potentially disrupt regulatory elements of the gene (Fig. 3A) (Young et al., 1996a). To determine whether sequences in the 5′ flanking region of the prairie vole OTR gene could direct OTR expression within a certain brain distribution, transgenic mice were created with the prairie vole OTR 5′ flanking region sequence directly in front of a *lacZ* reporter. These mice showed *lacZ* expression in brain regions in which prairie voles normally express OTR, suggesting region-specific OTR gene expression is at least partially controlled by *cis*-regulatory elements in the 5′ flanking region of the gene (Young et al., 1997b,c).

Genetic sequencing of the prairie and montane vole *avpr1a* gene revealed much larger differences in the *avpr1a* promoter sequence, concentrated in a large stretch of tandem repeats known as microsatellite DNA (reviewed in Hammock and Young, 2002; Lim et al., 2004a) (Fig. 3B). This species divergence in regulatory sequence is a functional polymorphism that has been shown to modulate gene expression in a cell-type-dependent manner (Hammock and Young, 2004). Transgenic mice were created carrying the prairie vole *avpr1a* gene and promoter region; these mice displayed a "prairie vole-like" V1aR distribution pattern, quite different from their wild-type littermates (as mentioned above) (Young et al., 1999). In addition, these mice showed increased affiliative behavior when infused i.c.v. with vasopressin, a response not seen in normal wild-type mice (Young et al., 1999). This strongly suggests that the species differences in social bond formation result from differences in V1aR distribution in specific brain regions, and this can be traced back to differences in the promoter sequence of the gene. Furthermore, prairie voles show individual variation in V1aR distribution throughout the brain, and this variation is correlated with length of the V1aR microsatellite and various aspects of social behavior (Hammock et al., 2005; Phelps and Young, 2003) (Fig. 3C). Individual prairie voles that have been specifically bred for long versus short homozygous alleles of the V1aR microsatellite show predictable V1aR brain patterns and performance on social behavior tests such as paternal care, social interest and partner preference formation (Hammock and Young, 2005) (Fig. 3C).

Implications for human bonding

Are there neurobiological correlates of social behavior in humans? Given the roles of oxytocin and vasopressin in social attachment in rodent and sheep models, one might expect to find abnormalities in these neuropeptides in patients with dysfunctional social relationships. Several studies in human patient populations do in fact support this hypothesis. One study found that autistic children had significantly lower levels of plasma oxytocin as compared to age-matched normal subjects (Modahl et al., 1998). Another recent study reported that orphaned children with adverse rearing environments had lower baseline vasopressin levels measured in urine than control children (Fries et al., 2005). However, an important caveat to remember is that peripheral levels of oxytocin and vasopressin do not necessarily reflect central levels (see Bartz and Hollander, 2006). One study has reported that oxytocin administration enhances some aspects of social cognition in autistic patients, suggesting that oxytocin may actually have some therapeutic value in disorders characterized by deficits in social cognition (Hollander et al., in press; Bartz and Hollander, 2006). In the spectrum of non-pathological human behavior, a recent study found that intranasal oxytocin administration increased feelings of trust in a game designed to test an individual's willingness to accept social risks (Kosfeld et al., 2005). A complementary fMRI study showed that intranasal oxytocin

administration reduced amygdalar activation, effectively uncoupling the amygdala from downstream brainstem targets of fear and anxiety (Kirsch et al., 2005).

Alterations in the vasopressin system may also be associated with human social behavior and autism. The vasopressin gene is closely linked to the oxytocin gene (both are located on 20p11–12); conceivably, a single critically placed mutation could influence the expression of both peptides (Fields et al., 2003). The prairie vole model of social attachment implicates V1aR activation in the brain during the formation of social bonds, and genetic polymorphisms exist in the *avpr1a* gene promoter between vole species that are associated with species differences in the ability to form pair bonds (Hammock and Young, 2002; Young et al., 1999). Monogamous vole species contain a microsatellite in the promoter of the *avpr1a* gene that regulates V1aR expression in the brain and thus leads to differences in social behavior (Hammock and Young, 2005). Interestingly, the human *avpr1a* gene has similar repetitive microsatellite elements in the promoter region, with polymorphisms in the number of tandem repeats. Up to sixteen different allelic forms at one microsatellite locus exist in the human population, and one of these alleles has been linked to autism using transmission disequilibrium analysis in three independent studies, although a functional variant still needs to be demonstrated before making a definitive link with autism (Kim et al., 2002; Wassink et al., 2004; Yirmiya et al., 2006). Sequencing the *avpr1a* gene across several non-human primate species also reveals differences in microsatellite elements within the promoter region that could contribute to differences in primate social structure (Hammock and Young, 2005).

However, to date there is still no consistent neurochemical, neurophysiological, or neuroanatomical abnormality observed across all autistic patients, and clinical heterogeneity of the disorder poses a monumental challenge to both scientists and clinicians. Current diagnoses and treatments are primarily behavioral in nature, and encompass wide variation and likely multiple convergent etiologies. Since the clinical phenotypes vary so greatly, it might also be useful to identify genes after parsing out subphenotypes of the disorder, such as by language deficits, savant skills, or primary deficits in social interactions. Based on animal models, if oxytocin and/or vasopressin are involved in the neuropathology of autism, we would hypothesize that abnormalities in these systems might account more specifically for the deficits in social interactions and processing, and perhaps less so for cognitive or language deficits. Multiple genes outside of oxytocin and vasopressin systems likely contribute to other subphenotypes, and it is likely that deficits in multiple genes, probably in a number of different combinations, are necessary to achieve the comprehensive phenotype (Bartz and Hollander, 2006).

In summary, animal models may provide valuable insights into neuropathology of complex psychiatric disorders by uncovering the neural control of normal social behaviors such as social motivation, social recognition and social bond formation. The current studies described here highlight the neuropeptides oxytocin and vasopressin in the regulation of these three levels. Comprehensive investigation of these neuropeptide systems, along with a number of other systems, may yield further insights into the genetic, cellular, and neural substrates underlying autism and other disorders of social behavior. The ultimate goal of this research to is identify potential therapeutic targets that may prove effective in the treatment of psychiatric disorders characterized by deficits in social motivation and social cognition.

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