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Using Transgenic Mouse Models to Study Oxytocin's Role in the Facilitation of Species Propagation

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Abstract

Oxytocin and its receptor are important for a wide range of effects, from social memory to uterine contractions. It is an evolutionarily well-conserved hormone that is particularly important in social and gregarious animals. Research on small mammals has yielded a rich literature on oxytocin's many functions. Recently a new tool has been created that has furthered our understanding of oxytocin's role in behavior: transgenic mice that lack either the ability to synthesize oxytocin or the oxytocin receptor itself. The study of these lines, while still in its infancy, is already bearing fruit and offers the promise of insight into some human disorders characterized by aberrant social behavior.

Keywords

Oxytocin; Oxytocin receptor; Transgenic animal; Social behavior

1. Introduction

Oxytocin is a nonapeptide hormone best known for its role in lactation and parturition. Oxytocin is composed of nine amino acids (Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–GlyNH₂) with a sulfur bridge between the two cysteines. It is very similar in structure to vasopressin. Both neuropeptides are evolutionarily well conserved across phyla (Acher et al., 1995; Caldwell and Young, 2006).

The oxytocin receptor is widely distributed throughout the brain. Sexual and species differences exist in the distributions of the oxytocin receptor, even within the same genus, and these differences are believed to explain certain variations in behavior. In rodents, oxytocin receptors are especially prominent in the olfactory bulb and tubercle, neocortex, endopiriform cortex, hippocampal formation (especially subiculum), central and lateral amygdala, bed nucleus of the stria terminalis, nucleus accumbens, and ventromedial hypothalamus (VMH) (Insel et al., 1991; Veinante and Freund-Mercier, 1997). In humans, expression is prominent in the basal nucleus of Meynert, the nucleus of the vertical limb of the diagonal band of Broca, the ventral part of the lateral septal nucleus, the preoptic/

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anterior hypothalamic area, the posterior hypothalamic area, the substantia nigra pars compacta, the substantia gelatinosa of the caudal spinal trigeminal nucleus and of the dorsal horn of the upper spinal cord, as well as in the medio-dorsal region of the nucleus of the solitary tract (Loup et al., 1989; Loup et al., 1991).

Oxytocin and oxytocin receptors are important for a wide variety of behaviors (see (Lee et al., 2009; Neumann, 2008; Neumann and Landgraf, 2008)). Many, if not most, of oxytocin's functions, from social interactions (affiliation, aggression) and sexual behavior to eventual parturition, lactation and maternal behavior may be viewed as specifically facilitating species propagation (Lee et al., 2009). In this review we will discuss how transgenic mice have furthered our understanding of oxytocin's role in affiliative behaviors. We will focus on a subset, the knockout (KO) mouse, as various transgenic lines expressing reporter constructs based on the oxytocin or oxytocin receptor gene have not yet lead to significant advances (Gould and Zingg, 2003; Katoh et al.; Young et al., 1990).

2. Oxytocin and Humans

In humans, oxytocin may enhance feelings of trust, strengthen memory for social stimuli and increase empathy, some aspects of which might be extremely difficult to model in mice. The fascinating studies presented in this section represent initial findings that will need replication and expansion. It is worth noting that many, if not most, of these studies were likely initiated in response to the KO mouse results.

Circulating levels of oxytocin are positively associated with mood and have a negative correlation with perceived stress in both men and women. In women, oxytocin is positively correlated with higher self-reported feelings of attachment on the Temperament and Character Inventory (Tops et al., 2007). Higher oxytocin levels are also correlated with more frequent physical contact between women and their spouses as well as lower blood pressure levels in pre-menopausal women (Light et al., 2005). Seltzer et al. (2010) have shown that children under stress show more rapid reduction in salivary cortisol levels after maternal contact or vocalizations that correlated with increased levels of urinary oxytocin. Linkage between the oxytocin receptor and affect and loneliness has also been reported (Lucht et al., 2009). The higher oxytocin levels seen in some post-partum mothers is associated with lower blood pressure and anti-stress effects (Light et al., 2000). Depressed women, who also show increased stress and disrupted social behavior, show irregular peripheral oxytocin release over time as compared to non-depressed controls (Cyranski et al., 2008).

The administration of oxytocin has been reported to increase pro-social feelings and behaviors. As adults, plasma oxytocin levels are positively correlated with feelings of affiliation to one's parents, and negatively correlated to reports of depression and anxiety (Gordon et al., 2008). Intranasal oxytocin increases subjective feelings of attachment to parental caregivers in adults reporting an insecure attachment (Buchheim et al., 2009).

Using a money transfer game, one group has shown that giving subjects intranasal oxytocin 50 min prior to playing a money transfer game causes them to give more money to a fictitious second player than those given a placebo, but only if subjects believed they were giving money to another person and not to a "project" (Kosfeld et al., 2005). Intranasal oxytocin maintains trusting behavior, even when subjects learn that their trust has been breached (i.e., the other player fails to return money 50% of the time (Baumgartner et al., 2008)). Similar to the trust game, intranasal oxytocin increases generosity (Subject 1 gives money to Subject 2 while taking into consideration the amount Subject 2 finds acceptable), but not overall altruism (Subject 1 gives to Subject 2 with no feedback from Subject 2 (Zak et al., 2007)). Oxytocin is not involved in just positive social behaviors, though: researchers (Shamay-Tsoory et al., 2009) demonstrated that subjects display increased levels of envy

following intranasal oxytocin. Oxytocin seems to particularly affect the ability to understand others' emotions, rather than affecting "trust" directly.

Intranasal oxytocin increases the amount of time spent gazing at the eye region of human faces (Guastella et al., 2008) and improves the ability to infer the mental state of others from social cues in the eye region (Domes et al., 2007). This increase in scanning the eye region may raise feelings of empathy by providing more social information and thus a greater subsequent understanding of social cues. Interestingly, oxytocin is involved not only in higher cognitive functions, but also in the perception of social stimuli: intra-nasal administration of oxytocin increases subjects' sensitivity to perceived biological motion (Keri and Benedek, 2009). Oxytocin appears to affect low-level attentional (eye movement) and perceptual mechanisms as well as sociability at a higher cognitive level,.

Oxytocin may also contribute to increased sociability by decreasing social anxiety. Lower plasma oxytocin levels have been linked to higher levels of psychological distress and less parental attachment (Gordon et al., 2008). Oxytocin decreases stress hormone release in humans (reviewed in (Legros, 2001) and appears to reduce amygdala activation in response to vague or threatening stimuli; it may also increase feelings of trust and affiliation by reducing amygdala activation induced by social or novel situations (Baumgartner et al., 2008; Meyer-Lindenberg, 2008). Intra-nasal oxytocin administration reduces amygdala activation in response to fearful/threatening scenes (Kirsch et al., 2005). Pre-stress intra-nasal administration of oxytocin blunts the social stress of speaking in front of an audience, a situation that increases reported feelings of stress as well as cortisol levels; this effect is enhanced by concomitant social support from a friend prior to the stressor (Heinrichs et al., 2003).

Oxytocin has also been shown to modulate learning about socially relevant stimuli. Subjects trained using the pairing of a mild shock with images of people directing their gaze towards or away from the observer show suppression of amygdala activity when given intranasal oxytocin; the suppression is even greater for images with direct rather than indirect gaze (Petrovic et al., 2008). Intranasal oxytocin also enhances learning in an associative task when social (happy/angry faces), but not non-social reinforcers (red/green circles) are used (Hurlemann et al.).

Recent work has led to speculation that abnormalities in oxytocin levels and/or receptor functioning may contribute to autism spectrum disorder. Plasma oxytocin levels may be lower in male autistic children than age matched controls (Modahl et al., 1998). More recently, the same group has replicated the original findings due to abnormal production of oxytocin peptide in the autistic group (Green et al., 2001). Associations between single nucleotide polymorphisms (SNP) in the oxytocin receptor and families with at least one autistic person in Chinese (Wu et al., 2005), Caucasian American (Jacob et al., 2007) and Israeli populations (Lerer et al., 2008) support the link between autism and oxytocin, though one study (Tansey et al.) found no oxytocin system associations in the brains of European Caucasians with autism.

Administering oxytocin appears to affect some behaviors that are features of autism. Infusion of Pitocin, a synthetic form of oxytocin, decreases repetitive behaviors in autistic adults (Hollander et al., 2003). Adults with autism or Asperger's syndrome also exhibit enhanced memory for affective speech after intravenous oxytocin administration (Hollander et al., 2007). In a recent study, adolescents with Asperger's or autistic disorder scored higher on a standardized test of the ability to read emotions from affective cues in the eye region following intranasal oxytocin (Guastella et al., 2010). This early evidence suggests a

potential linkage between autism and oxytocin and its receptor that remains a fascinating area to explore in humans.

3. The Study of Oxytocin and Oxytocin Receptor Knockouts

Oxytocin's role was originally thought to be limited to reproductive functions such as milk ejection and parturition. It has now been extended to include social, sexual and reproductive behaviors and bone formation (Lee et al., 2009; Tamma et al., 2009).

In 1996, two independent groups reported oxytocin KO mouse lines. Both lines showed normal parturition but failed milk ejection (Nishimori et al., 1996; Young et al., 1996), eliminating pup survival. Dams of oxytocin KOs exhibited relatively normal maternal behavior (Nishimori et al., 1996). However, additional studies revealed deficits in social memory (Ferguson et al., 2000), increased anxiety and stress responses to psychogenic and some physiological stimuli (Amico et al., 2004; Mantella et al., 2003), and increased intake of sucrose (Amico et al., 2005).

Oxytocin release is controlled, in part, by intracellular calcium (Ca^{2+}) stores (reviewed in (Ludwig and Leng, 2006)). CD38, a transmembrane glycoprotein, mobilizes Ca^{2+} and affects oxytocin secretion through formation of cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate. The generation of CD38 KO mice has allowed for investigation into the importance of proteins that regulate oxytocin secretion. CD38 KO mice show normal synthesis and storage of oxytocin in axon terminals but abnormally low oxytocin plasma concentrations, indicating abnormal release of oxytocin (Jin et al., 2007). Interestingly, these mice show deficits in social recognition similar to those of the oxytocin and oxytocin receptor KOs described below (see the section 5.1, social recognition). The detailed phenotypes of both oxytocin and oxytocin receptor KOs will be discussed later.

When given to humans, oxytocin is known to increase amnion prostaglandin, which can accelerate labor by inducing luteolysis and uterine contractions (Gross et al., 1998). Interestingly the prostaglandin synthetic enzyme cyclooxygenase (COX)-1KO mice show delayed onset of labor resulting in neonatal death whereas oxytocin KO mice demonstrate normal parturition. However, both oxytocin and COX-1 combined KO females exhibit labor at the normal time, meaning that the absence of oxytocin restores the onset of normal labor in COX-1 KO females (Gross et al., 1998). Further information on oxytocin and COX KOs can be found in Muglia (Muglia, 2000).

Two groups later generated and studied oxytocin receptor KO lines (Lee et al., 2008a; Takayanagi et al., 2005). Despite oxytocin's clear reproductive functions, oxytocin and oxytocin receptor KO mice show no clear deficits in mating and post-partum maternal behavior. These results may be explained by functional redundancy and developmental adaptation through the activation of the oxytocin system by other gene systems such as vasopressin and its receptors (Caldwell and Young, 2006; Lee et al., 2008b). In order to investigate this problem, conditional KO technology using the Cre-loxP system has been developed. Spatial and temporal gene inactivation can be achieved through Cre recombinase expression under tissue-specific promoters (Lewandoski, 2001).

Our line (Lee et al., 2008a) is conditional: the neomycin resistance cassette is flanked by *frt* sites that FLP recombinase recognizes to remove the cassette. This leaves a normally functioning oxytocin receptor gene (determined by *in vitro* receptor autoradiography) that could be inactivated in the presence of Cre recombinase (Lee et al., 2008a). We initially generated a total KO as well as a forebrain-specific KO (created by crossing with a calcium-calmodulin kinase 2 alpha (Camk2a) promoter-driven Cre recombinase transgenic line (see figure 1) (Dragatsis and Zeitlin, 2000)). This latter line shows deletion of oxytocin receptor

beginning at 21–28 days after birth, thus eliminating potential compensation for a deleted oxytocin receptor gene during early development (Lee et al., 2008a; Lee et al., 2008b). The total and forebrain KO mouse lines show different abnormalities in social memory (described in section 5.1), emphasizing the importance of the oxytocin receptor distribution and/or the timing of oxytocin receptor deletion (Lee et al., 2008a). However, in the absence of more specific Cre recombinase-expressing transgenics in the desired locations, the etiologies (i.e., anatomical contributions) of the differences remain to be discovered.

Additional studies using oxytocin KOs have found increased intake of sucrose in these mice (Amico et al., 2005) although this is not seen in our colony (unpublished observations) nor in the total oxytocin receptor KOs (Lee et al., 2008a). Mice from this line produce fewer ultrasonic vocalizations and have higher aggression levels than wild type littermates (Takayanagi et al., 2005). Also, late onset obesity is reported (Takayanagi et al., 2008), although we have not observed this phenotype in our oxytocin or oxytocin receptor colonies (unpublished observations). The oxytocin receptor-reporter mouse line, in which 50% of serotonergic neurons are positive for oxytocin receptor (Yoshida et al., 2009), has extended our understanding of the oxytocin system's possible function in the brain. The oxytocin receptor might play a role in the modulation of serotonin release, which is important in psychiatric diseases such as depression. These results suggest a possible role of the oxytocin system in metabolism under circumstances that have yet to be elucidated.

4. Oxytocin and Sex hormones

Oxytocin function is dependent on steroid hormones and gender (Francis et al., 2002). Both oxytocin and oxytocin receptor synthesis are dependent on estrogen. Rat and human oxytocin promoters harbor estrogen-response elements (ERE), which can activate oxytocin by binding with estrogen in the nucleus. However, DNA binding assays have shown that the interaction between estrogen and the oxytocin gene is more likely a DNA-independent mechanism in vivo (Stedronsky et al., 2002). Interestingly, osmotic stress increases poly (A) tail size in rats, which is important for mRNA stability, but only in gonadally intact animals (Carter and Murphy, 1989; Crowley and Amico, 1993), suggesting another signaling pathway that mediates the steroid hormone regulation of oxytocin expression. In estrogen receptor (ER)-alpha KO mice, estrogen induced oxytocin receptor binding is absent in the brain, whereas basal oxytocin receptor expression is similar to controls, suggesting ER-alpha is absolutely necessary for oxytocin receptor induction by estrogen (Young et al., 1998).

Estrogen-treated ovariectomized female rats show increased oxytocin receptor mRNA in the VMH (Bale and Dorsa, 1995). Oxytocin receptor mRNA expression is also elevated when estrogen levels are high during the estrous cycle in the VMH (Bale et al., 1995). The oxytocin receptor gene promoter harbors a complete ERE in mice, while rats and humans have low-affinity half palindrome EREs (Bale and Dorsa, 1997; Kubota et al., 1996; Sanchez et al., 2002). Estrogen receptors help control the oxytocin system, so Choleris and colleagues suggest a gene micronet involving the genes coding for ERalpha, ERbeta, oxytocin and oxytocin receptor as the regulatory basis of social recognition in the brain (Choleris et al., 2004).

5. Oxytocin Transgenic Lines and Social Behaviors

Oxytocin is critically important for many of the behaviors that allow for the propagation of the species. These behaviors include social recognition, aggression, sexual behavior and parental behavior.

5.1 Social recognition

The recognition of conspecifics is a crucial initial component of all social behavior. Without the ability to recognize individuals, it would be impossible to display the appropriate behavior, be it aggressive or affiliative. Oxytocin is important for social behavior in both males and females. The development of oxytocin and oxytocin receptor KO mice has been a crucial tool in furthering our understanding of the role oxytocin and the oxytocin receptor play in social recognition responses.

Male oxytocin KO mice fail to dishabituate to repeated presentations of ovariectomized females, an impairment that is rescued by the administration of oxytocin (Ferguson et al., 2000). The presence of oxytocin in the amygdala appears to play a key role in social recognition: wildtype (WT) but not oxytocin KO mice show activation of c-fos in the medial amygdala following exposure to a female (Ferguson et al., 2001). Interestingly, two independently derived lines of oxytocin KO mice fail to show any deficits in general sociability (Crawley et al., 2007), indicating that oxytocin is primarily involved in the memory component of social recognition.

A similar social recognition impairment has been described in another oxytocin receptor KO mouse line (Takayanagi et al., 2005). Unlike WT controls, these oxytocin receptor KO mice continue to investigate a previously presented 'familiar' female as if she were 'novel'. More recent work using our oxytocin receptor KO lines (both total and forebrain-specific oxytocin receptor KOs) has sharpened our understanding of role of the oxytocin receptor in social memory. We developed a novel social discrimination paradigm that uses group-housed male subjects that have not been sexually sated and singly housed female stimulus animals that are presented under corrals that allow for exploration but not contact. Our lab has shown that the oxytocin receptor is involved in fine social discrimination (intraspecies), but not broad, categorical (interspecies), social recognition: total oxytocin KO and oxytocin receptor KO males are unable to distinguish between individuals of the same strain but can discriminate between females from different strains, something the forebrain-specific oxytocin receptor KOs cannot do (Macbeth et al., 2009).

In females, estrogen appears to regulate oxytocin effects on social recognition. Specifically, ERalpha, ERbeta, and oxytocin KO mice all have very similar deficits in social recognition on the habituation–dishabituation test. In this type of test, a stimulus mouse is presented repeatedly at regular intervals until investigation time decreases below a predefined threshold (habituation). A novel mouse is then presented and if investigation times recover to levels that approach those of the initial exploration of the first mouse (dishabituation) the subject mouse has discriminated between the first and second mouse. All three of the above mentioned KO lines fail to habituate to a familiar animal or dishabituate with a novel animal, indicating that they fail to recognize the familiar mouse and are unable to discriminate between the familiar and novel mouse (Choleris et al., 2003). Similarly, in a social discrimination task, the three lines show either complete impairment (oxytocin and ERalpha KO mice) or partial impairment (ERbeta KO mice), indicating that all three genes are necessary to some degree for social recognition in females (Choleris et al., 2006).

Social deficits have also been reported in female transgenic mice using the Bruce effect, an accessory olfactory-based social memory task where housing a female with an unfamiliar male blocks pregnancy (Bruce, 1959) due to the chemosensory signals present in the new male's urine (Brennan, 2003; Dominic, 1966). While oxytocin KO and WT females display pregnancy block in response to an unfamiliar male, as expected, only oxytocin KO females display a social memory deficit by blocking pregnancy when a familiar male (their previous mate) is encountered after a 24 hour separation (Wersinger et al., 2008). Interestingly,

continuously paired oxytocin KO females do not pregnancy block, likely because with constant exposure, they do not have the opportunity to ‘forget’ their mates.

5.2 Aggression

Aggression is part of the complex repertoire of social behaviors that function to increase the likelihood of survival and reproduction. Aggressive behavior occurs in situations of competition (e.g., for food, mates or space), to establish hierarchy in a social group or in defense of altricial young. The type of situation that will elicit aggressive behavior, as well as the behavior display, depends on the species and sex of the animal studied (Miczek et al., 2007).

A clear picture is emerging from studies using transgenic mice. Initially, one line with inactivation of the oxytocin gene was shown to be mildly less aggressive than WT or HET controls, and showed no difference in anxiety behavior in an open field (DeVries et al., 1997). A different line of oxytocin KO mice displays increased aggressive behavior in the resident-intruder paradigm and decreased anxiety in the elevated plus maze (Winslow et al., 2000), but only in obligate KO mice born to homozygous KO parents (with the KO mice, along with their WT controls, cross-fostered to WT mothers). Non-obligates (KO's produced from HET-HET matings) show no reduction in anxiety and a small increase in aggression only on the third aggressive encounter (Winslow et al., 2000). This suggests that the effects on aggression and anxiety are due to the lack of oxytocin in the prenatal environment, or an interaction of genotype and the stress of cross-fostering. Elevated levels of aggression are also reported in oxytocin receptor KOs generated from HET-HET matings, consistent with the idea that a lack of prenatal activation of the oxytocin system results in increased adult aggression (Takayanagi et al., 2005).

Female mammals are most aggressive during the postpartum period. In rodents, the mother will attack an unfamiliar male introduced to the cage for several days after giving birth. This type of aggression, dubbed maternal aggression, is a complex behavior that is influenced by a variety of factors that have been extensively reviewed elsewhere (Lonstein and Gammie, 2002; Neumann, 2008; Veenema and Neumann, 2008). Currently studies of maternal aggression in transgenic lines are lacking and this area remains ripe for exploration.

Other aggressive behaviors have been studied in one line of female oxytocin KO mice for an extended period of time in a semi-naturalistic environment. Higher levels of food deprivation-induced aggression and intruder-induced aggression are seen in female oxytocin KOs than in WT mice (Ragnauth et al., 2005). Additionally, when testing the maternal behavior of virgin females, oxytocin KOs kill and cannibalize pups 100% of the time, compared to an infanticide rate of 17% (but no cannibalization) in WT females (Ragnauth et al., 2005). The authors conclude that in contrast to the relatively normal behaviors reported so far for oxytocin KOs reared and tested in standard shoebox housing, extended testing with naturalistic conditions may reveal more realistic roles for oxytocin (Ragnauth et al., 2005).

5.3 Sex Behavior

The acute administration of oxytocin has a facilitative effect on penile erections and sexual behavior in rodents. This may also be true in humans, as plasma oxytocin levels increase during sexual responses such as arousal and orgasm in both women and men (Carmichael et al., 1987). In men, plasma oxytocin is increased at ejaculation while vasopressin is raised during sexual arousal (Murphy et al., 1987). In women, plasma oxytocin levels are elevated 1-minute post orgasm when compared to baseline levels (Blaicher et al., 1999).

In rodents, moderate doses of oxytocin facilitate penile erections, whereas both lower and higher doses inhibit erection frequency (Argiolas et al., 1987). Oxytocin is unable to induce erections without testosterone, as castration eliminates erections even with administration of Oxytocin and apomorphine; erections are re-established with the co-administration of testosterone, however (Melis et al., 1994). In male rats, intracerebroventricular or intraperitoneal injections of oxytocin decrease the latency to ejaculate as well as the time between mating bouts (Arletti et al., 1985).

Additionally, oxytocin KO males produce normal litters when mated (Nishimori et al., 1996; Young et al., 1996), indicating apparent normal sexual behavior, and the KOs are still potent sexual triggers to hormone-primed females (Agmo et al., 2008). Therefore, other hormones and mechanisms are more critically involved in sexual behavior in males. For review, see (Argiolas and Melis, 2004; Argiolas and Melis, 2005; Carter et al., 1992).

In female rats, regulation of copulatory behavior occurs through interactions between estrogen or progesterone and oxytocin (reviewed in (Witt, 1995)). In ovariectomized female rats given estrogen/progesterone replacement, intracerebroventricular injections of oxytocin prior to pairing with males increase lordosis in response to mounting attempts (Arletti et al., 1985). Lordosis is also increased when either estrogen and oxytocin (Caldwell et al., 1986) or progesterone and oxytocin (Gorzalka and Lester, 1987) are administered.

The induction of female sexual behavior is mediated primarily by the medial preoptic area of the hypothalamus and the VMH (both regions underlie lordosis display; see (Kow and Pfaff, 1998)). Infusion of an oxytocin antagonist decreases lordosis bouts and duration (Benelli et al., 1994). Infusion of antisense oligodeoxynucleotides to the oxytocin receptor (to reduce its levels) into the VMH of females primed with estrogen blocks female receptivity to male rats (McCarthy et al., 1994). Similarly, infusion of the an oxytocin antagonist into the medial preoptic area of the hypothalamus prior to treatment with progesterone significantly decreases lordosis posturing, and increases duration of fighting with males (Caldwell et al., 1994).

As noted above, two independent labs each created a line of oxytocin deficit mice (Nishimori et al., 1996; Young et al., 1996). The females of both lines show generally normal sexual behavior, gestation and parturition; lactation is affected, however, and pups die shortly after birth unless milk letdown is rescued with oxytocin injections (Nishimori et al., 1996; Young et al., 1996). Oxytocin, however, mitigates “labor disruption due to circadian clock resetting” (Roizen et al., 2007). Thus oxytocin is essential for milk ejection, but not mating, parturition or milk production.

5.4 Parental Behavior

During pregnancy and when nursing pups oxytocin levels are increased in the ventral septum (Landgraf et al., 1991), supraoptic nucleus (Caldwell et al., 1987; Landgraf et al., 1992; Mezey and Kiss, 1991), and paraventricular nucleus of the hypothalamus (Caldwell et al., 1987). Oxytocin receptor expression is also significantly increased at parturition throughout the brain (particularly in the supraoptic nucleus, medial preoptic area of the hypothalamus, bed nucleus of the stria terminalis, olfactory bulb and amygdala), with oxytocin receptor expression returning to the levels seen in virgin rats by 12 h postpartum (Meddle et al., 2007). A likely reason for increased expression of oxytocin and the oxytocin receptor is to facilitate the onset and maintenance of maternal behavior, which is strongly regulated by oxytocin (see (Leng et al., 2008) for a recent review).

Oxytocin and oxytocin receptor KO mice show relatively normal maternal behaviors. However, a recent detailed study of maternal behavior in oxytocin KO and WT virgin

females towards foster pups indicates that fewer oxytocin KO females retrieve pups; those that do, retrieve fewer pups, and oxytocin KO females groom themselves and pups less than WT females (Pedersen et al., 2006). Similar deficits are seen in both oxytocin receptor KO virgin and postpartum females (Takayanagi et al., 2005).

Much less is known about the role of oxytocin in paternal behavior, likely due to the small number of species in which males care for young. To date there are no studies using transgenic mice to study the effects of paternal behavior, though there is evidence from the literature on prairie voles that oxytocin and vasopressin may act in concert to influence paternal behavior (Bales et al., 2004).

6. Conclusion

The use of transgenic mouse models has contributed greatly to our understanding of oxytocin and its receptor, as well as to what behaviors may be preserved through functionally redundant activation of the oxytocin system by other gene systems such as vasopressin and its receptors. This work has guided recent efforts to examine oxytocin's role in human social cognition as well. It is clear that this evolutionarily well-conserved neuropeptide plays a large role in facilitating the propagation of the species through its role in social memory and attachment, sexual and parental behavior, and aggression.

Abbreviations

Camk2a	calcium-calmodulin kinase 2 alpha
COX	cyclooxygenase
ER	estrogen receptor
ERE	estrogen-response element
HET	heterozygous
KO	knockout
VMH	ventromedial hypothalamus
WT	wildtype

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Figure 1.

Controlling the oxytocin receptor (OxtR) gene expression by Cre recombinase-mediated DNA recombination. The floxed oxytocin receptor gene allele is introduced by homologous recombination. The Camk2a promoter drives Cre recombinase (Cre) expression in transgenic mice relatively specifically in the forebrain (indicated by blue beta-galactosidase histochemistry). By crossing the floxed mice (showing normal oxytocin receptor *in vitro* receptor binding in upper left autoradiogram) with this Cre transgenic line, progeny are produced in which the conditional allele is inactivated only in the forebrain (lower autoradiogram). Therefore, oxytocin receptor expression still remains in other tissues, such

as the mammary glands. Cre recombinase recognizes two loxP sites (chevrons) and eliminates the floxed oxytocin receptor exons. Adapted from (Lee et al., 2008b).