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Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults

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Abstract

Oxytocin receptors (OXTR) in the nucleus accumbens (NAcc) promote alloparental behavior and partner preference formation in female prairie voles. Within the NAcc there is significant individual variation in OXTR binding and virgin juvenile and adult females with a high density of OXTR in the NAcc display an elevated propensity to engage in alloparental behavior toward novel pups. Over-expression of OXTR in the NAcc of adult female prairie voles using viral vector gene transfer facilitates partner preference formation, but has no effect on alloparental behavior, even though OXTR antagonists infused into the NAcc blocks both behaviors. We therefore hypothesized that long-term increases in OXTR signaling during development may underlie the relationship between adult OXTR density in the NAcc and alloparental behavior. To test this hypothesis, we used viral vector gene transfer to increase OXTR density in the NAcc of prepubertal, 21 day old female prairie voles and tested for both alloparental behavior and partner preference formation as adults. Consistent with a developmental impact of OXTR signaling, adults over-expressing OXTR from weaning display both increased alloparental behavior and partner preference formation. Thus, the relatively acute impact of elevated OXTR signaling in the NAcc on partner preference formation previously reported appears to be dissociable from the effects of longer term, developmentally relevant OXTR signaling necessary for modulating alloparental behavior. These results are consistent with the hypothesis that oxytocin can have both long-term "organizational" effects as well as acute "activational" effects on affiliative behaviors.

Keywords

Development; Oxytocin receptor; Pair bonding; Alloparenting; Monogamy

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Introduction

The neuropeptide oxytocin (OT) plays several important roles in reproductive physiology and behavior. Peripherally released OT coordinates the progression of parturition and lactation, while centrally released OT coordinates behavioral changes leading to the expression of maternal care and other prosocial behaviors (Kendrick et al., 1997; Kendrick et al., 1987; Pedersen and Prange, 1979). Within the central nervous system OT has an organizational effect that has life-long implications for adult social behavior and neuroendocrine function (Bales et al., 2007; Carter, 2003; Kramer et al., 2003; Lipschitz et al., 2003). Many facets of the OT neuroendocrine circuit are evolutionarily conserved; however, the distribution of oxytocin receptors (OXTR) is not. And inter- and intra-specific variation in the distribution of OXTRs has been linked to differences in social behavior (Insel and Shapiro, 1992a; Olazabal and Young, 2006b).

In contrast to traditional laboratory models like mouse and rat, prairie voles (*Microtus ochrogaster*) are genetically diverse microtine rodents that possess a rich repertoire of social behaviors, including pair bonding between mates and biparental care (Carter et al., 1995; Young and Wang, 2004). In the laboratory, females display remarkable individual variation in their display of partner preference formation and alloparental behaviors. Interestingly, this individual variation in alloparenting does not emerge to its fullest until adulthood. The majority of juvenile virgin females display nurturing behavior; however, only 40–60% of virgin adult females respond favorably to unrelated pups and engage in alloparental behavior, while the remainder either ignore or attack pups (Bales et al., 2004b; Lonstein and De Vries, 2000; Lonstein and De Vries, 2001; Olazabal and Young, 2005). This diversity in alloparental behavior may have an ethologically relevant function in the communal nest. In the wild, prairie voles maintain a varied and complex social structure. Approximately one-third of family units are single-mothers, one-third are male-female breeder pairs, and one-third are communal groups consisting of a breeder pair and several reproductively intact alloparents (Getz and Carter, 1996). In the laboratory, pups reared by single mothers show diminished spontaneous alloparental care and delayed partner preference formation (Ahern and Young, 2009), suggesting that early social experience may influence the development of socially relevant neuropeptide systems that can shape adult nurturing and attachment behaviors. The natural variation in social structure and behavior make the prairie vole an ethologically relevant laboratory model to study the developmental role of neuropeptide systems on organization of the social brain.

Within the prairie vole, OT promotes the display of alloparental behavior and partner preference formation. Clues on the neurobiological mechanisms underlying these effects came from comparisons across species. The socially monogamous prairie vole has higher densities of OXTR in the nucleus accumbens (NAcc), a region of the brain associated with reward and reinforcement, than do meadow voles, mice, and rats (Insel and Shapiro, 1992a; Olazabal and Young, 2006b). Prairie voles also exhibit higher levels of spontaneous maternal care than do meadow voles, mice, and rats (Insel and Shapiro, 1992b; Olazabal and Young, 2006b). Within prairie voles, OXTR density in the NAcc appears to drive individual differences in alloparental behavior, where the degree of spontaneous maternal care is positively correlated with OXTR density in the NAcc (Olazabal and Young, 2006a; Olazabal and Young, 2006b). When antagonists that block OXTR are injected into the NAcc of adult female prairie voles, they fail to display alloparental behavior (Olazabal and Young, 2006b).

OT also plays a role in regulating mating-induced partner preference formation within the prairie vole. The partner preference is a laboratory proxy for the social attachment made between two prairie voles following mating and cohabitation. In females, vaginocervical

stimulation during copulation is thought to cause the release of OT within the female's brain (Ross et al., 2009a). In the absence of mating, an infusion of OT into the brain during cohabitation with a male accelerates the formation of a partner preference (Williams et al., 1994). Paralleling the relationship between OXTR activation in the NAcc and alloparental behavior, when females are given an OXTR antagonist into the NAcc prior to mating, the development of the partner preference is blocked (Young et al., 2001).

These pharmacological blockade studies suggest that individual variation in accumbal OXTR density is a potential mechanism underlying diversity in social behavior. This relationship was directly tested using viral vector gene transfer to mediate over expression of OXTR in the NAcc of adult female prairie voles. Ross and colleagues (2009) found that increasing OXTR expression in adulthood increased partner preference formation but did not have an effect on alloparental behavior. Based on the inconsistency between the pharmacological blockade studies and the over-expression study, we hypothesized that in contrast to the acute effects of OXTR signaling on partner preference formation, increases in OXTR signaling during development may be necessary to impact adult alloparental behavior. To test this hypothesis, we used adeno-associated viral vector (AAV) gene transfer to over express OXTR during pubertal development. An AAV encoding the prairie vole OXTR gene was bilaterally infused into the NAcc of pre-pubertal female prairie voles (21 days of age), resulting in a significant elevation in OXTR binding, or a control virus expressing green fluorescent protein. Animals were then tested for alloparental behavior and partner preference formation in adulthood. We predicted that compared to controls, females with enhanced OXTR expression would show increased alloparental behavior and accelerated partner preference formation.

Materials and Methods

Animals

Prairie voles were housed in same-sex groups with 2–3 voles/cage from the time of weaning at 21d of age. Housing consisted of a ventilated 36 × 18 × 19 cm Plexigas cage filled with Bed-o-cobbs Laboratory Animal Bedding under a 14:10 h light/dark cycle (lights on 7:00AM–9:00PM) at 22°C with access to food (rabbit LabDiet) and water *ad libitum*. The prairie voles were obtained from our laboratory breeding colony that was originally derived from field-captured voles in Illinois. Subjects were weaned at 21d and immediately injected with virus. Stimulus animals for the partner preference tests were sexually experienced adult male prairie voles. Each male served as a “partner” and a “stranger” during the partner preference test (see below). Littermates were assigned to different treatment groups to control for variability within litters and within cages. All procedures were approved by the Emory University Institutional Animal Care and Use Committee.

Adeno-associated virus

The prairie vole CMV-OXTR and CMV-eGFP stock viruses used have been previously described (Ross et al., 2009b).

Viral vector infusion

Sterotaxic infusions were performed on 21-day-old females under isoflurane anesthesia in a Kopf stereotax fitted with an Ultra Micro Pump II (World Precision Instruments) and a 26 gauge Hamilton syringe. Females were injected bilaterally into the shell of the NAcc (anteroposterior +1.7mm, mediolateral ±0.9mm, dorsoventral −0.45mm) with 750 nl of either AAV containing the prairie vole oxytocin receptor (CMV-OXTR, n=14) or a control eGFP-expressing vector (CMV-GFP, n=11). Virus was infused at a rate of 5nl/sec. The syringe was left in place for 5 minutes following infusion to minimize diffusion up the

needle track. Sham operated females were anesthetized and had their scalps incised and sutured (n=11). Following surgery animals were group housed until time of partner preference testing.

Alloparental behavior testing

At 60d of age, prairie voles were tested for alloparental behavior. Testing occurred during the light cycle between 9:00 AM and 12:00 PM. Test animals were placed in a large clean cage (42.5 × 24 × 20 cm) and allowed to acclimate for 15 minutes followed by a 15-minute alloparenting testing session. Upon commencement of testing two pups (2–5d of age) were placed on one end of the cage. The latency to approach the pups, the amount of time spent grooming, hovering, and retrieving the pups, and the number of animals that attacked the pups were recorded. Testing was immediately stopped if the female attacked the pups. Animals were considered alloparental if they spent >30s huddling over the pups and >5s licking the pups without attacking as described previously (Olazabal and Young, 2005). A latency of 900s was assigned to animals that did not approach the pups for the purpose of statistical analysis.

Partner preference testing

One month following the alloparental testing (88 d of age) females were placed in a clean cage (28 × 17 × 12 cm) at 8:00 AM with a sexually experienced adult male for 6h. Females were not estrogen primed and therefore not immediately sexually receptive. At 2:00 PM females were examined vaginally to determine if mating had likely occurred. Females with vaginal redness and vaginal opening were considered to have mated. This method has been highly correlated with actual matings observed by video-recording (Ahern and Young, 2009). All test animals then underwent a 3h partner preference test, beginning at approximately 2:30pm. In a partner preference test, the experimental female is placed in a neutral chamber of a three-chambered apparatus, in which the male “partner” is tethered to one side of the chamber and a novel “stranger” male is tethered in the other (Williams et al., 1992). The experimental animal is free to move throughout the chambers and the time spent in close proximity to each male is recorded using an automated tracking system that tracks time spent in immobile contact, or “huddling” (Ahern et al., 2009). Animals were considered to have a partner preference if they spend twice as much time in immobile contact with the partner versus the stranger. Immediately following the 3h test, females were repaired with the same partner at approximately 6:00 PM for an additional 12h cohabitation. The females were then visually examined again to determine if mating had likely occurred and underwent a second partner preference test (18h total cohabitation). This two-stage paradigm was used to maximize our detection of a partner preference, since there is variability in the threshold time needed to form a partner preference. All tests were video recorded and analyzed *post hoc* for time spent in immobile contact with the stimulus animal by an automated software system.

Locomotor activity was measured during the partner preference test to determine if treatment affected general locomotor activity or anxiety-like behavior. All tests were video recorded and analyzed *post hoc* for the total distance traveled in the center cage by an automated software system as described above.

Tissue collection and processing

Following the behavioral experiments animals were decapitated following deep anesthetization with isoflurane. Brains were then collected and frozen on powdered dry ice. The brains were sectioned through the NAcc in 6 series at 20μm, on a cryostat, onto Fisher Frost-plus slides. Slides were stored at −80°C until used in receptor autoradiography.

OXTR receptor autoradiography

OXTR receptor autoradiography was used to assess OXTR binding in AAV-injected animals. Receptor autoradiography was performed as previously described with slight modifications (Ross et al., 2009b). Sections were removed from −80°C storage, allowed to air dry, dipped in 0.1% paraformaldehyde in PBS, pH7.4, and rinsed twice in 50 mM Tris buffer, pH 7.4, to remove endogenous OT. Next the tissue was incubated in 50 pM ^{125}I-OVTA (NEX 254050UC Perkin Elmer) for 1h. Unbound radioligand was removed by four washes in 50 mM Tris plus 2% $MgCl_2$, pH 7.4, and then dipped into dH_2O and air dried under a stream of cool air. Once dry, the slides were exposed to BioMax MR film (Kodak) for 72h. Five CMV-OXTR animals were grouped into an additional anatomical control group due to injection misses.

GFP visualization

Sections were removed from −80°C storage, allowed to air dry, and coverslipped using Krystalon (EMD Chemicals). GFP was visualized by fluorescent microscopy. Two CMV-GFP animals were excluded due to injection misses.

Statistical analysis

One-way ANOVAs were run on alloparenting behaviors. Dunnet's test was used for *post hoc* analysis when significant effects were detected. All animals were categorized as either alloparental or nonalloparental. Fisher's exact test was used to compare the proportion of alloparental females across groups and the proportion of females that mated across groups. Data from the partner preference test were analyzed using two-way ANOVAs in which stimulus (partner or stranger) and treatment were factors. The Holm-Sidak test was used for *post hoc* pairwise comparisons when a significant interaction effect was detected. When only a main effect of stimulus animal was found Students *t*-test were used to compare time in contact with the partner and stranger within each treatment group. Bonferroni corrections for the level of significance were made to minimize risk of type I errors due to multiple comparisons. Data collected for locomotor activity was analyzed using a one-way ANOVA with treatment as the independent factor.

Animals were divided into one of four groups. The experimental group, CMV-OXTR, received bilateral injections of the OXTR expressing virus into the nucleus accumbens (n=9). The first control group (CMV-GFP) received bilateral accumbal infusions of a viral vector expressing the GFP gene (n=9). The second control group (CMV-OXTR miss) consisted of animals bilaterally injected with CMV-OXTR whose viral injections were inadvertently placed outside the nucleus accumbens (n=5); these animals were regrouped, *ex post facto*, in the analysis after the completion of behavioral testing. The third control group (Sham) were anesthetized, had their scalps incised, and sutured (n=11).

Results

Alloparental behavior

There was a significant treatment effect on latency to approach pups ($F(3,31)=5.58$, $p=0.0035$, one-way ANOVA) and time spent licking and grooming the pups ($F(3,31)=9.52$, $p=0.0001$, one-way ANOVA). *Post hoc* tests revealed that CMV-OXTR females displayed a shorter latencyto approach pups (102.2 ± 69.1s, $p=0.043$) than did CMV-OXTR miss (448.8 ± 161.2), CMV-GFP controls (437.3 ±103.2) and sham females (381.9 ± 114.2) (Fig. 1A). Similarly, *post hoc* tests revealed that CMV-OXTR females spent significantly more time licking and grooming pups (559.1 ± 114.8s, $p=0.006$) than did the CMV-OXTR miss (40.6 ± 27.0), CMV-GFP controls (198.4 ± 45.5) and sham females (195.1 ± 34.9) (Fig. 1B). The proportion of CMV-OXTR injected female prairie voles meeting the categorical criteria for

displaying alloparental behavior (9/9) did not differ from the CMV-OXTR miss (2/5), CMV-GFP control (6/9) or sham voles (5/11) (Fisher's exact test, p=0.09) (Fig.1C). Zero OXTR-CMV, one CMV-OXTR miss, three CMV-GFP control, and 4 sham females, which were categorized as nonalloparental, attacked the pups.

Cohabitation and mating

Mating facilitates partner preference formation in prairie voles; however, in the absence of mating a minimum 24 hour cohabitation period is typically required for the formation of a partner preference, although this varies across laboratories (Carter et al., 1995). Our visual method of mating analysis showed that the proportion of individuals that mated after 6h (mated/total: CMV-OXTR 1/9, CMV-OXTR miss 1/5, CMV-GFP control 0/9 or sham voles 2/11) and 18h (CMV-OXTR 8/9, CMV-OXTR miss 4/5, CMV-GFP control 7/9 or sham voles 10/11) did not differ between groups (Fisher's exact test p=0.625 and p=0.55, respectively). Therefore, we did not exclude animals that mated from any of the groups.

Partner preference behavior

Following the 6h cohabitation there were no main effects of treatment ($F(3,55)=0.50$, $p=0.600$, two-way ANOVA) or time spent with stimulus animal ($F(3, 55)=1.054$, $p=0.309$, two-way ANOVA). However, there was a significant interaction effect ($F(3,55)=5.982$, $p=0.025$). *Post hoc* tests revealed that CMV-OXTR injected females spent significantly more time in immobile contact with the partner than with the stranger ($p=0.028$), but none of the control groups showed this preference (Figure 2A). Following the 18h cohabitation there was a significant main effect of stimulus animal ($F(3,66)=5.758$, $p=0.019$, two-way ANOVA), but no other main effects ($F(3,66)=1.17$, $p=0.329$) or interactions ($F(3,66)=1.23$, $p=0.307$) were detected. To determine which animals spent more time in contact with partners over strangers Students *t*-tests were performed with Bonferonni corrections of the p-value. CMV-OXTR injected females preferentially spent more time with their partners over the strangers ($p=0.003$, Student's *t*-test, Bonferroni level set at $p<0.01$) (Figure 2B) while none of the control groups showed this preference.

Locomotor activity

Locomotor activity did not differ between treatment groups following a 6h ($F(3,29)=1.076$, $p=0.374$, one-way ANOVA) or 18h ($F(3,29)=0.261$, $p=0.852$, one-way ANOVA) cohabitation period.

OXTR and GFP expression in the NAcc

Receptor autoradiography was done to determine placement of AAV injection and to verify that the CMV-OXTR vector resulted in increased OXTR binding compared with controls. Representative OXTR binding in the NAcc of control sham and CMV-GFP injected prairie voles are shown in Fig. 3A, B. Distinct elevations in OXTR binding were detected in the NAcc of CMV-OXTR injected prairie voles (Fig. 3C). Distinct elevations in OXTR binding were detected outside of the NAcc in CMV-OXTR miss prairie voles (Fig. 3D).

Discussion

In the socially monogamous prairie vole there is remarkable individual variation in alloparental behavior and this variation is positively correlated with activation of OXTR densities in the NAcc (Olazabal and Young, 2006a). Pharmacological blockade of those receptors in the prairie vole eliminates both alloparental behavior and partner preference formation (Olazabal and Young, 2006a; Young et al., 2001). Increasing OXTR expression in the NAcc of adult prairie voles increases partner preference formation but does not increase

alloparental behavior suggesting (1) that other brain systems may be involved in generating this behavioral diversity (Ross et al., 2009b), or (2) that longer term developmental effects of OXTR signaling may be important in the display of affiliative behaviors in adult virgin females.

In this study we used viral vector gene transfer to increase OXTR density in the NAcc of prepubertal, 21 day old female prairie voles and tested for alloparental behavior and partner preference formation in adulthood. Consistent with a developmental impact of OXTR signaling, adults over-expressing OXTR from weaning display both increased alloparental behavior and partner preference formation. Thus, the relatively acute impact of elevated OXTR signaling in the NAcc on partner preference formation previously reported by Ross et al. (2009b) is dissociable from the effects of longer term, developmentally relevant OXTR signaling necessary for modulating alloparental behavior.

These results are consistent with the hypothesis that OT can have both acute “activational” effects as well as long-term “organizational” effects on affiliative behaviors. For pair bonding, *in vivo* microdialysis experiments have shown that mating stimulates OT release in the NAcc of female prairie voles, leading to direct OXTR activation (Ross et al., 2009a) and facilitating partner preference formation. In contrast, alloparental behavior may be more sensitive to OXTR activation during development. Within prairie voles there is significant individual variation in the degree to which females engage in spontaneous alloparental care. The majority of juvenile prairie voles display alloparental care; however, as females reach puberty only about 40–60% will spontaneously engage in alloparental care (Bales and Carter, 2003b; Lonstein and De Vries, 1999; Olazabal and Young, 2005). Juveniles with higher densities of OXTR signaling in the NAcc may have long-lasting neurochemical changes that increase the probability of displaying alloparental behavior as they become adults.

Previous studies in prairie voles have shown that early exposure to OT, via exogenous or endogenous sources, can have life-long, sexually dimorphic implications for social behavior and neuroendocrine function. For example, a single treatment with OT 24h after birth facilitated the formation of a partner preference in males but not females (Bales and Carter, 2003b) while treatment with OTA decreased alloparenting and partner preference formation in males but not females (Bales et al., 2004a). While these initial studies found marked behavioral changes only in males, subsequent studies have shown that females display a dose-dependent response. These effects were not linear and both increased and decreased the propensity for females to display alloparenting and partner preference behaviors (Bales et al., 2007). Females are also more likely to be alloparental as adults if they remain in the natal nest after weaning, with continued exposure to parents being a more critical factor than previous pup exposure (Lonstein and De Vries, 1999). The presence of the father in the natal nest also increases the amount of time juveniles of both sexes engage in alloparental behavior toward their younger siblings and their propensity to form a partner preference (Ahern et al., 2011; Wang and Novak, 1992). These studies suggest that the level of OXTR activation during development, perhaps when the pups are interacting with their parents and siblings, is critical for shaping affiliative behavior in adulthood, and response to endogenous OT release. In the present study, OXTR expression was elevated upon weaning from the parental unit. However, it is possible that continued interaction with siblings might provide sufficient OT stimulation to produce long-term effects on affiliative behavior. The mechanism by which OT produces these long-term effects is unknown, but these data suggest that like gonadal steroids, OT can have both activational and organizational effects on social behavior (Bales and Carter, 2003a; Carter, 2003; Kramer et al., 2007; Lipschitz et al., 2003).

The findings that overexpression of accumbal OXTR in prepubertal female prairie voles enhances subsequent hormone activation of female affiliative behaviors in adulthood is consistent with the extended organizational-activational hypothesis of hormone driven sex differences in brain and behavior (Phoenix et al., 1959; Schulz et al., 2009). This extended hypothesis includes a second stage of steroid dependent neuronal organization during adolescence (Schulz et al., 2009). Data is beginning to emerge suggesting that ovarian hormones during the neonatal and adolescent periods actively feminize strategies for food defense (Field et al., 2004) and digestive responses to metabolic signals (Swithers et al., 2008) in rats. Previous studies support the hypothesis that OT expression during neonatal development has an organizational effect on the expression of estrogen receptor alpha (Kramer et al., 2007; Perry et al., 2009; Yamamoto et al., 2006) and the serotonin system (Eaton et al., 2011) that are age-dependent and site specific. It is becoming increasingly clear that neuropeptides interact with steroids to regulate behavior and ovarian hormones clearly organize some female behaviors during the adolescent period. Thus, it is possible that OT release during adolescence has an organizational effect with life-long implications for social behavior and neuroendocrine function.

Direct OT action on the accumbal OXTR population seems to be of critical importance for the expression of affiliative behaviors in the female prairie vole, and developmental activation of OXTR signaling leads to differences in the expression of these behaviors. It is possible that the enhanced affiliative behaviors observed in this study were due simply to longer OXTR activation, rather than to increased OXTR expression during a developmental window. As compared to the study done by Ross and colleagues (2009), OXTR activation was increased for an additional 11 days before behavioral testing was initiated. However, it should be noted that this is unlikely given the short time difference (39 days in the present study vs 28 days in Ross et al., 2009b) as well as the association between the OT system and organization of the CNS on social behavior observed in previous studies. It should also be noted that while the studies here support a developmentally important role for the OT system and the NAcc in regulating affiliative behavior in the female prairie vole, these behaviors are undoubtedly regulated by a larger circuitry involving multiple brain regions and neurochemicals.

In humans, OT has been shown to modulate social behavior, including interpersonal trust, eye gaze, facial memory, and emotion perception (Ditzen et al., 2009; Domes et al., 2007; Guastella et al., 2008; Kosfeld et al., 2005). Independent groups have reported that intranasal OT enhances some aspects of social functioning in Autism Spectrum Disorders (ASD) (Andari et al., 2010; Guastella et al., 2010; Hollander et al., 2007). Furthermore, one recent study found that peripheral *OXTR* gene is hypermethylated in ASD subjects, which could result in decreased OXTR expression (Gregory et al., 2009). These findings make the OT system an attractive candidate for neural modulation of human social relationships as well as a potential therapeutic target for the treatment of psychiatric disorders associated with disruptions in social behavior. Our studies suggest that the effects of longer-term, more chronic applications targeting the OT system may influence a different suite of behaviors than acute adult treatment. It is possible that earlier stimulation of the OT system, via endogenous release or through external administration, would have a wider, more enduring effect on social behavior.

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Highlights

- Oxytocin receptor density in the NAcc of female prairie voles is positively correlated with spontaneous alloparental behavior and partner preference formation.
- Enhanced oxytocin receptor signaling in the NAcc during adulthood enhances partner preference formation but has no effect on spontaneous alloparental behavior in adults.
- Enhanced oxytocin receptor signaling in the NAcc during pre pubertal development enhances partner preference formation as well as spontaneous alloparental behavior in adults.
- Oxytocin receptor activation during development plays an important role in the expression of affiliative behaviors in adulthood.

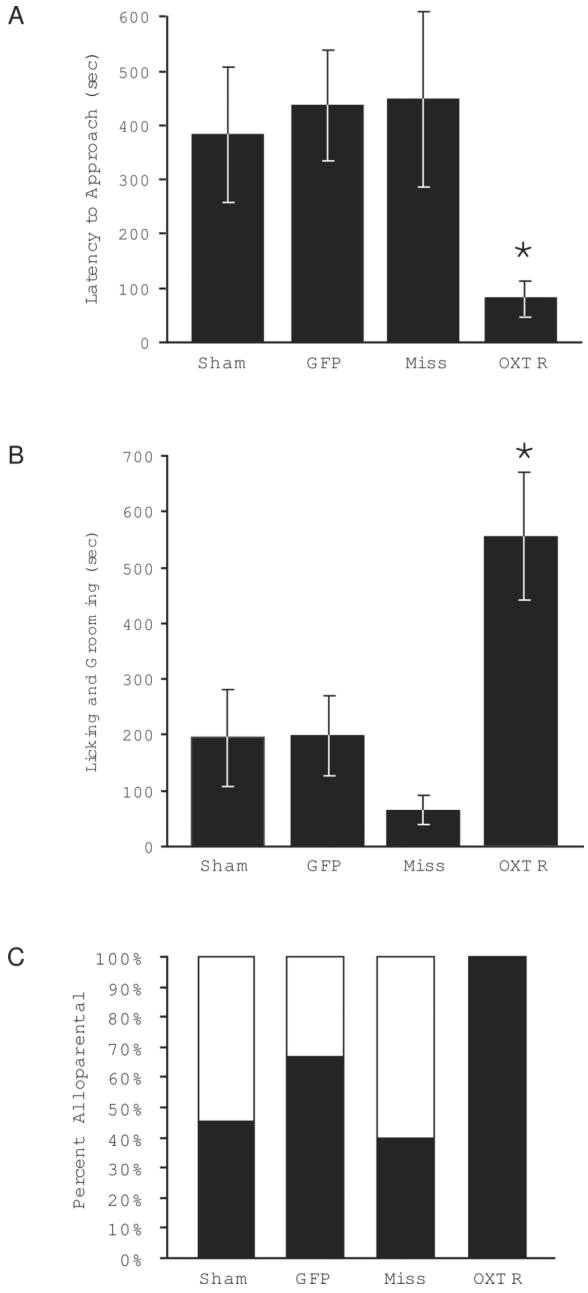

Figure 1.

Alloparental behavior in Sham, CMV-GFP, CMV-OXTR miss, and CMV-OXTR female prairie voles treated on day 21. The control group "Sham" had their scalps incised and sutured. A second control group "CMV-GFP" received bilateral accumbal infusions of a virus expressing the GFP gene. The experimental group "CMV-OXTR" received bilateral injections of the OXTR expressing virus into the nucleus accumbens while the third control group "CMV-OXTR miss" received bilateral injections inadvertently placed outside the nucleus accumbens. ***A,*** The CMV-OXTR group had a shorter latency to approach pups as compared to the Sham, CMV-GFP, and CMV-OXTR miss treatment groups. ***B,*** The CMV-OXTR group engaged in licking and grooming for a longer duration than did the Sham, CMV-GFP, and CMV-OXTR miss treatment groups. ***C,*** There was no effect of treatment on the proportion of females in each treatment group that displayed alloparental behavior. The

dark bars represent the percentage of animals that were categorized as alloparental, while the light bars represent the number of females that attacked or ignored pups. Asterisk represents a p-value less than 0.05. Data are represented as mean ±SEM.

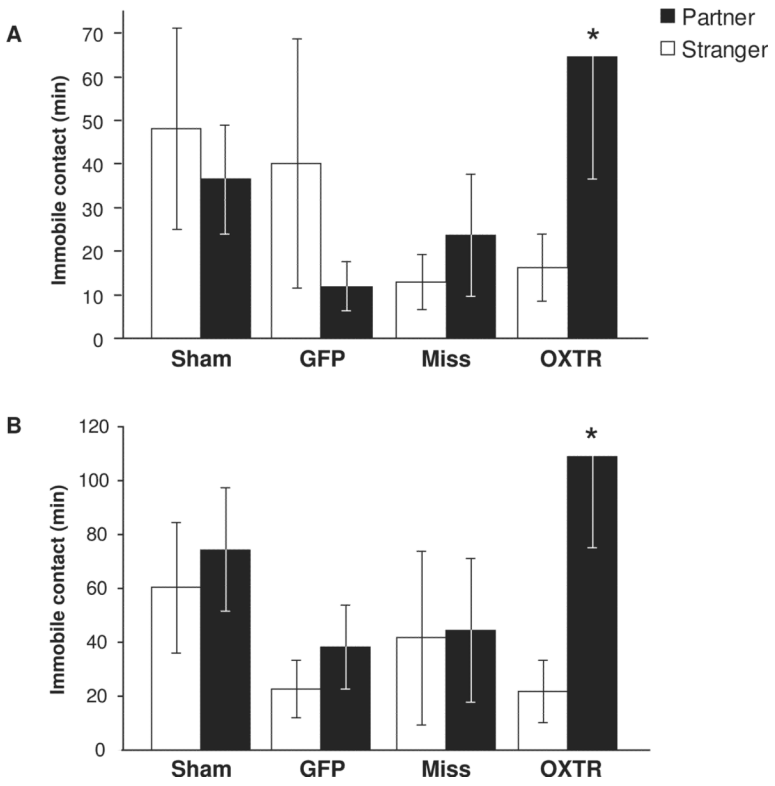

Figure 2.

Partner preference behavior in Sham, CMV-GFP, CMV-OXTR miss, and CMV-OXTR female prairie voles treated at 21 days old. ***A,*** After a 6h cohabitation period only the CMV-OXTR group displayed a significant preference for the partner over the stranger. ***B,*** After a cumulative 18h cohabitation period, only the CMV-OXTR group displayed a significant preference for the partner over the stranger. Asterisk represents a p-value less than 0.05. Data are represented as mean ±SEM.

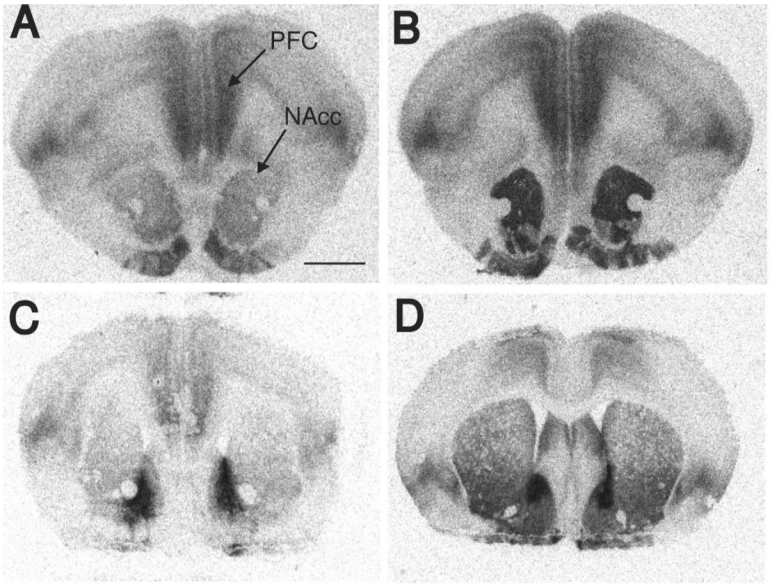

Figure 3.
OXTR receptor autoradiography illustrating the density of OXTR binding in Sham (A), CMV-GFP (B), and CMV-OXTR (C) treated female prairie voles. Animals injected with CMV-OXTR placements outside the NAcc were placed in a third control group, CMV-OXTR miss (D). There was significant individual variation in the density of OXTR binding in the NAcc across all treatment groups as illustrated by comparing A and B. This is in contrast to the prefrontal cortex (PFC), where there was little variation in OXTR binding density. Females infused with the CMV-OXTR viral vector had elevated OXTR binding the NAcc relative to controls. Scale bar, 1 mm.