



Oxytocin facilitates fidelity in well-established marmoset pairs by reducing sociosexual behavior toward opposite-sex strangers



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Summary Behavioral strategies that facilitate the maintenance of social bonds are critical for the preservation of high-quality social relationships. Central oxytocin (OT) activity modulates the behavioral features of socially monogamous relationships in a number of mammalian species (including marmoset monkeys), and plays a vital role in the behavioral maintenance of long-term social relationships. Two distinct variants of OT have been identified in some New World primates (including marmosets; Lee et al., 2011). The marmoset variant of the oxytocin ligand (Pro⁸-OT) is structurally distinct from the consensus mammalian variant of the oxytocin ligand (Leu⁸-OT), due to a proline substitution at the 8th amino-acid position. The goal of the present study was to determine if treating marmosets with Pro⁸-OT, relative to treatments with Leu⁸-OT, control saline, or an OT antagonist, had modulatory effects on the behavioral maintenance of long-term social relationships in marmosets. Treatment with the Pro⁸ variant, but not the Leu⁸ variant, of OT facilitated fidelity with a long-term partner by reducing time spent in close proximity with an opposite-sex stranger. However, this facilitative effect of Pro⁸-OT on proximity behavior manifested itself differently in male and female marmosets, such that females preferred to interact socially with their partner rather than a stranger when treated with Pro⁸-OT, while males spent less time in close proximity with both their partner and a stranger when treated with Pro⁸-OT. Furthermore, treatment with Pro⁸-OT, but not Leu⁸-OT, significantly delayed the expression of sexual solicitation behavior toward an opposite-sex stranger in both male and female marmosets, but had no effect on sociosexual behavior directed toward a long-term partner. These results suggest that the OT system is highly

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involved in reducing fidelity-threatening behaviors in well-established marmoset pairs, and that the effects were only produced by species-specific OT ligands.

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1. Introduction

Behavioral strategies that facilitate the maintenance of social bonds are critical for the preservation of high-quality social relationships between adult mates. The well-known functions of oxytocin (OT) in parturition (Blanks and Thornton, 2003) and lactation (Caruolo, 1971) have been recognized for decades; there is continuing interest in the neuromodulatory role of OT in social relationships (Insel et al., 2001). OT has been implicated in a suite of prosocial behaviors (MacDonald and MacDonald, 2010), particularly for its facilitative role in social bonding (Young and Wang, 2004). OT not only facilitates maternal bonding behaviors (Kendrick, 2007), but also promotes affiliative behavior and a partner-preference in adult sociosexual relationships (Lim and Young, 2006).

Monogamous social relationships are defined by a pervasive and reciprocal sociosexual preference for a long-term partner over an opposite-sex stranger, as well as high levels of sociosexual behavior between pair-mates (Hawkes, 2004). Accordingly, the differential behavioral response (i.e., prosocial, sexual, and/or aggressive) to an opposite-sex stranger is a good measure of attachment and fidelity to a pair-mate (Gubernick and Nordby, 1993). Fidelity to a long-term mate is threatened when one, or both, member(s) of a long-term pair spend time in close proximity, and engage in sociosexual behavior, with an opposite-sex stranger. OT has been shown to regulate fundamental mechanisms underlying mate fidelity. Human males in a monogamous relationship, but not single men, who received intranasal OT kept a greater distance on approach of an attractive woman (Scheele et al., 2012). Furthermore, central infusion of OT reduced the duration required to form a stable pair-bond in the socially monogamous prairie vole (*Microtus ochrogaster*; Williams et al., 1994). Blocking endogenous OT activity diminished a partner-preference and inhibited pair-bond formation in prairie voles (Cho et al., 1999). Thus, the OT system plays a critical role in the formation and maintenance of long-term monogamous relationships.

Marmosets (genus *Callithrix*) belong to a clade of New World primates (marmosets and tamarins) with a diverse set of socially monogamous mating strategies, including high levels of sociality with a pair-mate (Schaffner et al., 1995), avoidance of a potential mate in the presence of a pair-mate (Evans, 1983; Inglett et al., 1990), and aggressive responses to potential same-sex rivals (Ross et al., 2004). Like the socially monogamous prairie vole, marmosets form and maintain sociosexual relationships between males and females (Digby, 1995; Schaffner et al., 1995). However, unlike prairie voles, which display consistently strong sociosexual preferences for a long-term partner over an opposite-sex stranger (Wang and Aragona, 2004), marmosets display a flexible pattern of sociosexual preferences, similar to those displayed by humans (Buss and Schmitt, 1993), including a

willingness to interact with a potential mate in the absence of a pair-mate (Evans, 1983; Inglett et al., 1990). For these reasons, the marmoset is an excellent translational primate model for the study of social-bond maintenance.

There is evidence from both correlational and experimental studies that OT plays a role in modulating social interactions in marmosets and tamarins. Urinary levels of OT co-vary with social behavior in male and female cotton-top tamarins (*Saguinus oedipus*), with variance in female OT levels associated with grooming behavior and mutual physical contact with a partner, and variance in male OT levels associated with sexual behavior (Snowdon et al., 2010). Thus, greater expression of affiliative and sexual behavior, which contributes to the quality of long-term social relationships, is associated with higher OT levels. In marmosets, paternal food sharing behavior was influenced by experimental treatment with an OT agonist. Marmoset fathers treated with OT displayed an increase in food sharing behavior with offspring (Saito and Nakamura, 2011). The development of sociosexual relationships in marmosets was also altered by pharmacological manipulation of the OT system in the first three weeks of cohabitation. Blocking endogenous OT activity in both males and females reduced measures of social proximity and food sharing with a new pair-mate (Smith et al., 2010). However, treatment with an OT agonist had a limited effect, and only subtly augmented marmoset sociality during the early period of cohabitation. To date, however, no studies have systematically manipulated the OT system in well-established pairs.

OT amino-acid sequences are highly conserved among placental mammals (Acher, 1980). However, two distinct variants of OT have been identified in some New World primates (including marmosets; Lee et al., 2011). The marmoset variant of the OT ligand (Pro⁸-OT) is structurally distinct from the consensus mammalian variant of the OT ligand (Leu⁸-OT), due to a proline substitution at the 8th amino-acid position. This amino-acid substitution results in a substantially altered structural geometry of the OT ligand relative to the consensus mammalian variant; changes in structure that may affect OT–OT receptor (OTR) binding and downstream signaling. Therefore, the limited effect of the consensus mammalian OT agonist on marmoset sociality (Smith et al., 2010) may be a consequence of the distinct amino-acid structure of the OT ligand in marmosets.

The goal of the present study was to evaluate the impact of pharmacological manipulations of the OT system on patterns of sociosexual behavior and partner/stranger preferences in well-established marmoset pairs. Further, we tested OT ligand-specificity in modulating social behavior by administering both Pro⁸-OT and Leu⁸-OT. If OT promotes mate fidelity, then treatment with OT agonists should increase preferential associations with a partner over a stranger, and decrease sexual solicitation toward an opposite-sex stranger, while treatment with an OT antagonist (OTA) should decrease preferential associations

with a partner, and increase sexual solicitation toward an opposite-sex stranger. Furthermore, if structural changes in the OT ligand alter OT signaling properties, then treating marmosets with Pro⁸-OT, but not Leu⁸-OT, should increase preferential associations with a partner and decrease sexual solicitation behavior directed toward an opposite-sex stranger. Thus, the goal of the study was to determine if treatment with Pro⁸-OT, relative to treatments with Leu⁸-OT, and an OTA, had modulatory effects on sociosexual behavioral responses to an opposite-sex stranger in the presence of a long-term partner in marmosets.

2. Method

2.1. Subjects

We tested six adult male and six adult female white-tufted ear marmosets (*C. jacchus*), housed at the Callitrichid Research Center (CRC) at the University of Nebraska – Omaha. Four unfamiliar marmosets of each sex were also required to test the subjects' interactions with opposite-sex strangers in each of the four treatment periods. Animals were 3.2 ± 0.2 (mean \pm SEM) years of age at the start of the study, and were kept in large indoor wire-mesh enclosures (1.0 m \times 2.5 m \times 2.0 m), equipped with a sleeping hammock, natural branches for climbing and various enrichment materials. Visual access was restricted between enclosures, but auditory and olfactory cues were not. Colony rooms at the CRC were maintained on a 12 h:12 h light: dark cycle and at a temperature range between 19°C and 22°C. For all dietary and husbandry protocols please refer to [Schaffner et al. \(1995\)](#).

All males were surgically vasectomized a minimum of seven months prior to the initiation of the study. We vasectomized males to avoid pregnancy and the subsequent presence of young offspring, which could potentially complicate male–female relationships. All females received a 0.15 mL intra-muscular injection of cloprostenol (Estrumate®), a synthetic prostaglandin analog, three days prior to each treatment period to synchronize females' ovarian cycles throughout the duration of the experiment by inducing luteolysis (i.e., the structural and functional degradation of the corpus luteum; [Summers et al., 1985](#)). The University of Nebraska – Omaha/University of Nebraska Medical Center Institutional Animal Care and Use Committee evaluated and approved all procedures: Protocol #: 12-099-12-FC.

2.2. Partner-preference testing

After eight weeks of cohabitation, which has been shown to be a more than adequate duration of time to allow for significant establishment of a stable social bond in marmosets ([Ágmo et al., 2012](#)), male and female marmosets underwent partner-preference testing. Proximity behavior, as well as sociosexual, aggressive/territorial, and communicative behaviors were observed during the partner-preference test. Partner-preference testing took place 30 min post-treatment between the hours of 0900 h–1030 h. The treated marmoset was placed at one end of a T-shaped partner-preference apparatus, and was allowed to interact with either an opposite-sex stranger or long-term partner

Table 1 Experimental design.

Condition	Male	Female
Marmoset OT agonist	Pro ⁸ -OT	–
Consensus OT agonist	Leu ⁸ -OT	–
OT antagonist	OTA	–
Placebo control	Vehicle	–
Marmoset OT agonist	–	Pro ⁸ -OT
Consensus OT agonist	–	Leu ⁸ -OT
OT antagonist	–	OTA
Placebo control	–	Vehicle

Note: Diagram identifying treatment conditions for males and females including: marmoset oxytocin variant agonist (Pro⁸-OT), consensus mammalian oxytocin variant agonist (Leu⁸-OT), oxytocin antagonist (OTA), placebo control (vehicle), and untreated partners (–).

(through 2 cm \times 2 cm wire mesh barrier), or no social preference, for 20 min. A different opposite-sex stranger was used for each treatment period. The long-term partner and opposite-sex stranger were placed on opposite ends of the T-shaped apparatus in a counterbalanced order, and visual contact between the partner and stranger was obscured via an opaque barrier. Social preference for either a long-term partner or an opposite-sex stranger was determined by measuring close proximity (within 30 cm), as well as sociosexual behavior (i.e., open-mouth displays). Open-mouth displays are expressed as an invitational behavior (i.e., proceptivity) and during copulation (i.e., receptivity; [Kendrick and Dixson, 1983](#)). Grooming and mating behaviors between the subject and partner/stranger were precluded due to the wire-mesh barrier. Observers were trained to achieve a level of proficiency ($\kappa > 0.90$) on scoring proximity, sociosexual, aggressive/territorial, and communicative behaviors. All focal animal observations were recorded using Stopwatch+ software (Emory University) by directly observing marmoset behavior during the partner-preference test. Marmosets were habituated to the partner-preference apparatus by allowing them to explore the apparatus without any social stimuli over a series of three 30-min sessions that took place in the two-week period prior to the first treatment period. This partner-preference testing procedure was repeated for each of the treatment periods over a period of five months (refer to [Table 1](#) for experimental design).

2.3. Drug treatments

2.3.1. Intranasal administration of OT agonists

Marmosets were administered 25 IU (50 μ g/100 μ L saline solution) of an OT agonist or vehicle, which yielded a dose of 150 μ g/kg. The dose was determined based on previous primate literature ([Parker et al., 2005](#); [Smith et al., 2010](#)). Marmosets were administered either Pro⁸-OT (Anaspec Corp, California), Leu⁸-OT (synthesized by Maurice Manning, University of Toledo), or vehicle, 30 min prior to the partner-preference test, via intranasal administration during a brief (~3 min) restraint. Intranasal administration was conducted using a 100- μ L Eppendorf pipette to administer 50- μ L of solution to each nostril drop-wise (30 s between each nostril), and is a relatively well-tolerated, non-invasive method of administration.

Peptides administered intranasally are quickly absorbed into the bloodstream via the nasal passage, and appear to bypass the blood–brain barrier (BBB) to access the central nervous system (CNS) via the olfactory bulb and the maxillary branch of the trigeminal nerve (MacDonald and MacDonald, 2010; Bethlehem et al., 2013). The neuropeptides OT and arginine–vasopressin (AVP) are transported to the CNS and accumulate in the cerebrospinal fluid (CSF) in humans (Born et al., 2002; Striepens et al., 2013) and rhesus macaques (Chang et al., 2012). In rats and mice, OT levels were increased in microdialysates from the hippocampus and amygdala, and plasma, 30–60 min after intranasal administration (Neumann et al., 2013). Circulating levels of OT persisted for up to, but no more than 7-h in humans (Van IJzendoorn et al., 2012). These results suggest that intranasal administration rapidly upregulates OT levels in the brain and plasma during the timeframe of behavioral testing, and that OT clears the system several hours after testing.

2.3.2. Oral administration of OT antagonist

Marmosets were treated with 20 mg/kg OTA, or vehicle, 90 min prior to the partner-preference test, via oral administration in a preferred food treat. The OTA (L368,899; provided by Dr. Peter Williams, Merck & Co., Inc.) is readily absorbed by the bloodstream after passage through the digestive system (Thompson et al., 1997), penetrates the CNS after peripheral administration, and accumulates in areas of the limbic system (Boccia et al., 2007).

Each member of the pair was treated individually during each treatment period in a counterbalanced order. Furthermore, the sequence of treatments among each individual was counterbalanced over the length of the study. Marmosets were treated every other day for ten days (days 1, 3, 5, 7, 9). Social behavior between pair-mates was observed in their home environment on days 1, 3, 5, and 7 (data not shown). Partner-preference testing took place on day 9. There was an 11–13 day washout period between each treatments period.

2.4. Data analysis

To assess possible social preferences for interacting with a long-term partner, an opposite-sex stranger, or spending time alone, we calculated several derived variables. The proportion of time spent in proximity (average duration/visit) with a stranger, relative to the time spent in proximity to both the partner and stranger, was subtracted from the proportion of time spent in proximity (average duration/visit) with the partner, relative to the time spent in proximity to both the partner and the stranger [i.e., $((\text{Partner})/(\text{Partner} + \text{Stranger})) - ((\text{Stranger})/(\text{Partner} + \text{Stranger}))$]. This measure yields a score from -1.0 to $+1.0$, where $+1.0$ indicates a strong social preference for a long-term partner. We used similar calculations to create variables for the preference to spend time in proximity with a partner vs. alone and for the preference to spend time in proximity with a stranger vs. alone. The effect of OT treatment on behavior exhibited during the partner-preference test was evaluated using several mixed-model ANOVAs, with OT treatment condition and sex as factors. If main effects or

interactions were significant, post hoc comparisons were made using Fisher's least significant difference. All alpha levels were set at $p < 0.05$.

3. Results

OT treatment influenced the proportion of time spent in close proximity to a long-term partner or an opposite-sex stranger differentially for male and female marmosets, as indicated by the significant interaction between OT treatment and sex [$F(3, 30) = 3.13, p = 0.04, \eta^2 = .24$]. Treatment with Pro⁸-OT profoundly reduced female marmosets' preference to spend time in close proximity with a stranger rather than their partner. Female marmosets treated with Pro⁸-OT spent proportionately less time in close proximity with an opposite-sex stranger vs. a long-term partner, relative to when they were treated with vehicle [$t(5) = 2.74, p = 0.04$] or Leu⁸-OT [$t(5) = 4.36, p = 0.007$] (Fig. 1A). OT treatment did not significantly alter proximity preferences for a partner vs. a stranger in male marmosets, as they spent roughly equivalent amounts of time with both an opposite-sex stranger and their long-term partner in all treatment conditions (Fig. 1B).

OT treatment also influenced the proportion of time spent in close proximity to a long-term partner or alone differentially for male and female marmosets, as indicated by the significant interaction between OT treatment and

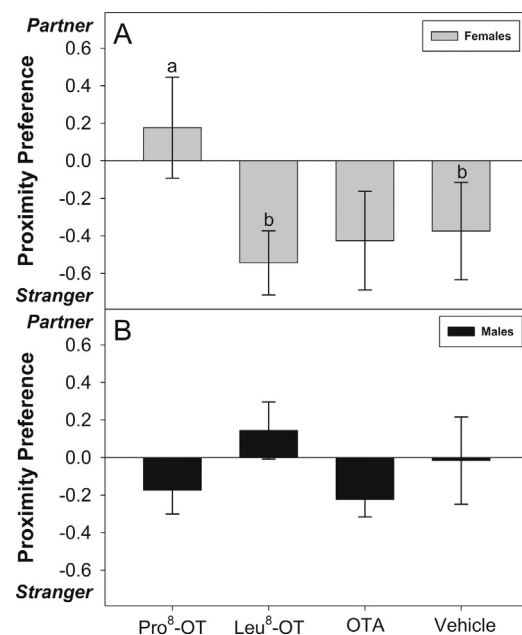


Figure 1 Preference: partner vs. stranger. Preference scores (\pm SEM) for spending time in close proximity with a long-term partner or an opposite-sex stranger, expressed as a scale from -1 to $+1$. A positive score indicates a greater proportion of time (per bout) spent in close proximity with a partner vs. a stranger, while a negative score indicates a greater proportion of time (per bout) spent in close proximity with a stranger vs. a partner. Data are expressed as a function of OT treatment: marmoset oxytocin variant agonist (Pro⁸-OT), consensus mammalian oxytocin variant agonist (Leu⁸-OT), oxytocin antagonist (OTA: L368,899), and vehicle in (A) treated females and (B) males. Letters indicate significant differences ($a > b$ at $p < 0.05$).

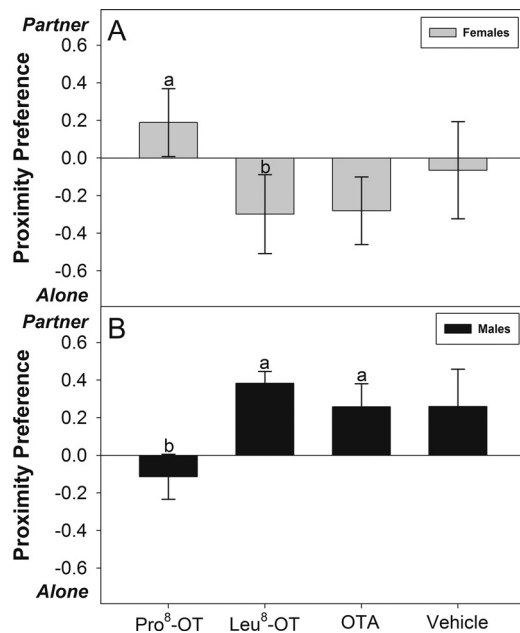


Figure 2 Preference: partner vs. alone. Preference scores (\pm SEM) for spending time in close proximity with a long-term partner or alone, expressed as a scale from -1 to $+1$. A positive score indicates a greater proportion of time (per bout) spent in close proximity with a partner vs. alone, while a negative score indicates a greater proportion of time (per bout) spent alone vs. with a partner. Data are expressed as a function of OT treatment (as indicated in Fig. 1) in (A) treated females and (B) treated males. Letters indicate significant differences ($a > b$ at $p < 0.05$).

sex [$F(3, 30) = 3.50, p = 0.027, \eta^2 = .26$]. Female marmosets treated with Pro⁸-OT spent proportionately less time alone vs. in close proximity with a partner, relative to when they were treated with Leu⁸-OT [$t(5) = 4.10, p = 0.009$], but not relative to treatments with vehicle or OTA [$p > 0.05$] (Fig. 2A). Interestingly, male marmosets treated with Pro⁸-OT spent proportionately less time in close proximity with their partner vs. alone, relative to when they were treated with Leu⁸-OT [$t(5) = 4.65, p = 0.006$] or OTA [$t(5) = 4.19, p = 0.009$], but not relative to vehicle [$p > 0.05$] (Fig. 2B).

OT treatment also influenced the proportion of time spent in close proximity to an opposite-sex stranger or alone in marmosets, as indicated by the significant main effect of OT treatment [$F(3, 30) = 2.99, p = 0.046, \eta^2 = .23$]. There was no significant effect of sex, or an interaction between OT treatment and sex, on the preference to spend time alone or with a stranger. Vehicle-treated marmosets spent a greater proportion of time in close proximity with a stranger vs. alone [$t(11) = 4.29, p = 0.001$]. Marmosets treated with Leu⁸-OT [$t(11) = 2.36, p = 0.038$] or OTA [$t(11) = 3.08, p = 0.01$] also spent a greater proportion of time in close proximity with a stranger vs. alone. However, marmosets treated with Pro⁸-OT spent proportionately less time in close proximity with an opposite-sex stranger vs. alone, relative to when they were treated with vehicle [$t(11) = 2.59, p = 0.025$] or OTA [$t(11) = 3.54, p = 0.005$] (Fig. 3). Overall, males engaged in significantly more locomotion (i.e., total frequency of proximity to partner/stranger/alone) than

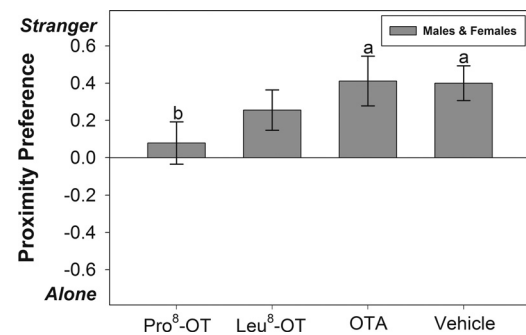


Figure 3 Preference: stranger vs. alone. Preference scores (\pm SEM) for spending time in close proximity with an opposite-sex stranger or alone, expressed as a scale from -1 to $+1$. A positive score indicates a greater proportion of time (per bout) spent in close proximity with a stranger vs. alone, while a negative score indicates a greater proportion of time (per bout) spent alone vs. with a stranger. Data are expressed as a function of OT treatment (as indicated in Fig. 1). Letters indicate significant differences ($a > b$ at $p < 0.05$).

females, as indicated by the significant main effect of sex [$F(1, 10) = 8.61, p = 0.015$].

Marmosets engaged in sexual-solicitation behavior toward their partner or an opposite-sex stranger differentially due to OT treatment, as indicated by the main effect of treatment [$F(3, 30) = 4.19, p = 0.014, \eta^2 = .30$]. There was no significant effect of sex, or an interaction between OT treatment and sex, on the expression sociosexual behavior. OT treatment did not have a significant effect on the latency to open-mouth display toward a long-term partner (Fig. 4A). However, OT manipulations markedly altered

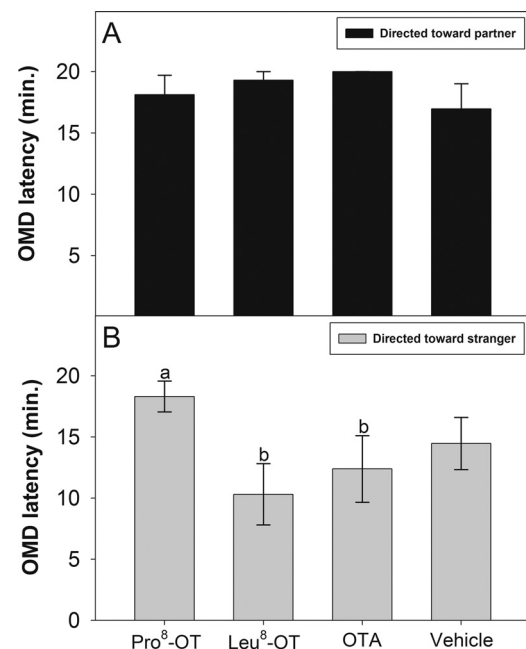


Figure 4 Sexual-solicitation behavior: latency. Latency (\pm SEM) to open-mouth display (OMD) at either (A) a long-term partner or (B) an opposite-sex stranger, expressed as a function of OT treatment (as indicated in Fig. 1). Letters indicate significant differences ($a > b$ at $p < 0.05$).

the latency to open-mouth display toward an opposite-sex stranger. Marmosets treated with Pro⁸-OT took significantly longer to open-mouth display toward an opposite-sex stranger, relative to when they were treated Leu⁸-OT [$t(11)=3.05$, $p=0.011$] or OTA [$t(11)=2.21$, $p=0.049$] (Fig. 4B). Marmosets treated with Pro⁸-OT tended to take longer to open-mouth display to a stranger, relative to when they were treated with vehicle [$t(11)=1.89$, $p=0.08$]. Aggressive/territorial and communicative behaviors were not influenced by OT treatment or sex.

4. Discussion

Central oxytocin activity appears to modulate behavioral strategies that facilitate the preservation of long-term social relationships by reducing the expression of socio-sexual behavior toward opposite-sex strangers. Treatment with the Pro⁸ variant, but not the consensus mammalian variant, of OT facilitated fidelity with a long-term partner by decreasing time spent in close proximity with, and increasing the latency to exhibit sexual-solicitation behavior toward, a stranger. However, this facilitative effect of OT on fidelity manifested itself differently in male and female marmosets, such that females preferred to interact socially with their long-term partner rather than an opposite-sex stranger when treated with Pro⁸-OT, while males spent less time in close proximity with both their partner and a stranger when treated with Pro⁸-OT. Accordingly, increased levels of centrally available species-specific OT agonists significantly reduced fidelity-threatening behaviors in marmosets by enhancing a preferential association with a partner and simultaneously decreasing associations with a stranger in females, but not males.

Preference for a partner in marmosets and tamarins differs from the prairie vole model (Wang and Aragona, 2004) in the sense that it is labile, both within and among species, and tends to be strongly influenced by social context. Male saddle-back tamarins (*Saguinus fuscicollis*) typically interact with a partner more than a stranger, while females display no preference (Epple, 1990). Male and female and golden-lion tamarins (*Leontopithecus rosalia*) typically interact with a stranger more than a partner under conditions when the partner's visual access to the stranger is blocked or the partner is absent, but not when the partner has visual access to the stranger (Inglett et al., 1990). Male, but not female, marmosets (*C. jacchus*) typically display sexual solicitation behavior to a stranger in the absence of their mate. Furthermore, in the presence of their long-term partner, both male and female marmosets reduced the expression of sexual behavior and engaged in more aggressive behavior toward an unfamiliar conspecific (Evans, 1983). Male and female marmosets (*C. penicillata*) interacted more with a stranger than a newly paired partner after 24 h of cohabitation. However, after three weeks of cohabitation, male and female marmosets interacted equally with both a partner and a stranger (Smith et al., 2010). The results of these studies suggest that social context plays a crucial role in partner/stranger preferences in marmosets and tamarins, and that length of cohabitation strongly influences partner/stranger preferences by reducing interactions with an opposite-sex stranger.

The current study showed that in well-established marmoset pairs, females spent proportionately more time in close proximity with a stranger over their partner under control conditions. This pattern is consistent with the finding that when an individual's partner is present, but visual access between the partner and stranger is occluded, marmosets will interact with an opposite-sex stranger, as noted above. However, female marmosets treated with the Pro⁸ variant, but not the consensus mammalian variant, of OT had a reduced preference to interact with an unfamiliar potential mate. This suggests that one effect of increased OT may be to make it less likely for individuals in long-term social relationships to form social bonds with opposite-sex strangers.

Female marmosets treated with Pro⁸-OT spent proportionately less time in close proximity with a stranger. This finding could be interpreted in two subtly distinct ways related to social recognition. Social recognition (i.e., ability to differentiate a familiar individual from a stranger, and remember a previously encountered conspecific) is an essential skill for an individual living within a social group (Ross and Young, 2009). OT is highly involved in the regulation of social recognition, by facilitating social memory and selective attention to socially relevant information through action in the medial amygdala (Petrovic et al., 2008; Samuelsen and Meredith, 2011). Furthermore, social recognition and familiarity strongly influence partner/stranger preferences (Cheetham et al., 2008). Thus, OT may either be modulating (1) the specific social distinction between a long-term partner and an opposite-sex stranger, or (2) the less sophisticated social distinction between a familiar "in-group" member and an unfamiliar "out-group" member. Thus, our finding that Pro⁸-OT reduced female marmosets' preference for spending time in close proximity with a stranger may be interpreted as a shift away from a socially unfamiliar stranger and toward a socially familiar partner.

In addition to the facilitative effect of Pro⁸-OT on a partner-preference, female marmosets also displayed altered preferences to either spend time alone or interact socially with either their partner or a stranger under OT treatment. Female marmosets treated with Pro⁸-OT increased preferential associations with their partner over spending time alone and simultaneously decreased their preference for a stranger over spending time alone. This behavioral pattern is consistent with findings in prairie voles that OT increases preferential associations with a pair-mate (Williams et al., 1994; Cho et al., 1999). Thus, treatment with the Pro⁸ variant, but not the consensus mammalian variant, of OT facilitated a behavioral strategy to increase preferential associations with a partner over a stranger, and to reduce time spent in close proximity with a stranger in favor of spending more time alone.

Interestingly, males spent an equal amount of time in close proximity with their partner and a stranger in all treatment conditions, which is in contrast to the reduction in stranger-preference under Pro⁸-OT treatment in females. This sex difference suggests that the OT system in males may be differentially insensitive to an OT intervention in the context of modulating social distance between a partner and a stranger. In prairie voles, intracerebroventricular injections of OT induced a partner-preference in both males and females (Cho et al., 1999), whereas subcutaneous injections

of OT only induced a partner-preference in females (Cushing and Carter, 2000), suggesting that route of administration may have an effect on the differential behavioral response between males and females. Despite the similar phylogeny and socially monogamous mating structure of marmosets and humans, each appears to have different behavioral responses to OT interventions. In humans, males treated with OT perceived their pair-mates face as more attractive than the face of an opposite-sex stranger, and experienced increased neural activity in the branches of the brain's reward system (i.e., nucleus accumbens, ventral tegmental area; Scheele et al., 2013). Unlike the positive effect of OT on perceived partner attractiveness in human males, OT appears to decrease the attractiveness of opposite-sex strangers in female marmosets, but does not appear to have a strong effect on male marmosets' perceptions of their pair-mate or an opposite-sex stranger. Alternatively, male and female marmosets may have different behavioral responses to their partner and a stranger during OT interventions due to sex-specific consequences of extra-pair sexual encounters (i.e., increased potential for offspring vs. potential deleterious effects on current social relationship), or levels of anxiety (male marmosets exhibited greater levels of locomotion during partner-preference testing).

Treatment with Pro⁸-OT did not increase male marmosets' preference for their partner over a stranger. The limited effect of OT treatment on partner/stranger preferences in male marmosets may indicate that the OT system simply influences male sociosexual behavior less so than female behavior. This may be explained by the potential influence of the related neuropeptide, AVP, on partner/stranger preferences in males. In addition to the critical role of AVP in partner-preference formation (Winslow et al., 1993), it is also highly involved in other male-typic behavior, including aggression and courtship (Goodson and Bass, 2001). Numerous studies in prairie voles have implicated the OT system as potentially more important for sociosexual bond development in females, and the AVP system as more important for sociosexual bond development in males (Young and Wang, 2004). More likely, both the OT system and the AVP system are highly involved in modulating partner/stranger preferences and the regulation of sociosexual behavior between pair-mates in both males and females. In the current study both male and female sexual solicitation behavior was strongly influenced by OT treatment, but OT only affected female's partner/stranger preferences. Thus, one potential avenue for future research is to examine these context-specific behavioral responses during a social preference paradigm under AVP treatment conditions.

Multiple neuroendocrine systems may be interacting to modulate sociosexual preferences, which may help explain the sex-specific behavioral responses to an opposite-sex stranger in marmosets. The OT system may be interacting with the hypothalamic–pituitary–adrenal (HPA) axis to modify sex-specific behavior responses. Novel environments and distinct social contexts generate anxiety-like behavior and concurrent activation of the HPA axis in marmosets (Smith and French, 1997), and since OT is a well-known anxiolytic agent (Smith and Wang, 2012), an alternative interpretation of the differential behavioral responses during the partner-preference test is that treatment with Pro⁸-OT may be acting on the HPA axis to modify anxiety-like

responses, as opposed to selectively acting on social neural circuits. Although, the results of the current study do not fall in line with the hypothesis that OT is anxiolytic. Instead, treatment with Pro⁸-OT reduced a preference for interacting with a potentially anxiety-inducing stimulus (i.e., opposite-sex stranger) in favor of a socially familiar partner in female marmosets. Thus, future investigations may find it prudent to use anxiolytic agents as controls to determine if OT manipulations are acting specifically on social neural circuits or on more generalized anxiety circuits (Churchland and Winkielman, 2012).

Additionally, the steroid/peptide theory of social bonds (Van Anders et al., 2011) provides a series of predictions for the integration of the OT system and the hypothalamic–pituitary–gonadal (HPG) axis, which is known to play a role in the formation of sociosexual relationships (Burnham et al., 2003; Van Anders and Goldey, 2010). To the extent that high levels of testosterone are associated with sociosexual competition, and low levels of testosterone are associated with nurturing physical contact with a partner (Van Anders et al., 2011), we might expect OT responses to be antagonistic with testosterone responses in some behavioral contexts and facilitative in other contexts. Marmosets (particularly males) treated with OT that experience increases in testosterone, when exposed to an opposite-sex stranger, might be receiving two opposing biological signals that influence their sociosexual preferences. Thus, an examination of the interaction between the OT system with the HPA and HPG axes may provide valuable insight into sex-specific behavioral responses to an opposite-sex stranger in well-established social relationships.

Not only did Pro⁸-OT modulate the duration of time spent in proximity with a partner or a stranger, but it also altered sociosexual interactions with a stranger. Vehicle-treated marmosets engaged in sexual-solicitation behavior significantly more quickly toward a stranger than their partner. While treating marmosets with the consensus mammalian variant of OT did not alter these patterns, treating marmosets with Pro⁸-OT significantly increased the latency to engage in proceptive sexual displays toward a stranger. In at least one wild population of marmosets, extra-pair sexual behavior has been reported during intergroup interactions (Digby, 1999), potentially as a way to avoid inbreeding or as a way females migrate to another group (Bicca-Marques, 2003); yet there is no evidence to suggest that these extra-pair encounters diminish the quality of the bond with their long-term partner (Digby, 1999). Since infidelity appears to occur under normative conditions, one mechanism that may lead to the persistence of the long-term bond between pair-mates is via endogenous OT release upon reunion with a pair-mate. In chimpanzees, excreted OT levels were higher following grooming bouts with a bonded partner (regardless of genetic relatedness) than a non-bonded partner (Crockford et al., 2013). Thus, socio-physical interactions with a pair-mate following extra-pair encounters may facilitate the preservation of long-term bonds via OT system activation.

In the current study, partner-directed sexual behavior with a long-term partner was virtually absent; yet, stranger-directed sexual behavior occurred relatively quickly under control conditions. It is quite interesting that treatment with OT did not increase partner-directed sexual behavior, as the

OT system has been implicated in female sexual receptivity (Cushing and Carter, 2000), as well as sexual arousal and orgasm (Behnia et al., 2014) during interactions between pair-mates. There did appear to be a ceiling effect in sexual solicitation latency toward the partner, suggesting that, relative to vehicle, it would be nearly impossible to observe an increase in latency to open-mouth display under OTA treatment. The results of the current study suggest that treatment with Pro⁸-OT reduced the likelihood of engaging in a sexual encounter by reducing stranger-directed sexual-solicitation behavior. Consequently, one effect of increased central OT activity is the pronounced reduction of fidelity-threatening behaviors, by reducing both the time spent in close proximity with a stranger and reducing sexual solicitations toward a stranger.

Pro⁸-OT had clear modulatory effects on social preferences for a partner vs. a stranger. However, treatment with an OTA did not significantly alter partner-stranger preferences. This finding is surprising because treatment with an OTA has been shown previously to reduce proximity, huddling, and food sharing behavior with a recently formed partner in marmosets. Furthermore treatment with an OTA reduced the frequency, and increased the latency, to establish contact with a partner before engaging with a stranger, after three weeks of cohabitation and continuous treatment (Smith et al., 2010). One possible explanation for the limited influence of an OTA in the current study is that endogenous OT release may not have been sufficiently stimulated due to the lack of socio-physical interactions with the partner or stranger. Another more likely explanation for the limited influence of an OTA is that well-established social relationships are less sensitive to OTA interventions, relative to the more dynamic sociosexual preferences of newly formed social bonds. Alternatively, acute treatment of an OTA in the current study may not have been sufficient to elicit the same reductions in sociality seen previously and modulate partner/stranger preferences in well-established social relationships.

5. Concluding remarks

Female marmosets treated with the Pro⁸ variant of OT spent significantly more time away from a stranger, than vehicle-treated females. This suggests that in the presence of a pair-mate, female marmosets find novel social stimuli less attractive when treated with centrally available OT, and therefore display a reduced preference for a stranger. Treatment with the Pro⁸ variant, but not the consensus mammalian variant, of OT led to a decrease in more salient behaviors, such as sexual-solicitation behavior toward a stranger, in both males and females. Therefore, there seems to be a distinct sexual dichotomy in the effect of the OT system on the expression of sociosexual behavior in marmosets, which suggests there may be some underlying differences in physiology of the social and/or anxiety neural circuits.

This was the first study to determine if treatment with the Pro⁸ variant of OT has a functionally significant effect on fidelity and sociosexual preferences. We were able to elucidate critical underlying neuroendocrine mechanisms that influence maintenance of social relationships. Treatment

with the Pro⁸ variant, but not the consensus mammalian variant, of OT reduced time spent in close proximity, and the expression of sociosexual behavior, with an opposite-sex stranger. Thus, treatment with centrally available OT significantly reduced fidelity-threatening behaviors in well-established adult sociosexual relationships as a means to maintain a vital social bond with a long-term pair-mate.

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Conflict of interest

None declared.

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