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Oxytocin regulates reunion affiliation with a pairmate following social separation in marmosets

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Abstract

While separation from significant social partners produces a host of neurobiological and behavioral perturbations, including behavioral distress and increased glucocorticoid production, positive social interactions upon reunion are critical for the reestablishment of normative relationship dynamics and the attenuation of the biobehavioral stress response. The hormone oxytocin has critical and pervasive roles in reproductive and behavioral processes across the lifespan, and plays a particularly prominent role in social bonding. In this study, we examined the extent that oxytocin modulates interactions with a pairmate following separation challenges that varied in both social context (isolation; separation) and duration (long; short), in marmosets. We demonstrated that the impact of pharmacological manipulations of the oxytocin system on the expression of affiliation upon reunion depended on both the context and duration of the separation challenge. Specifically, marmosets treated with an oxytocin antagonist spent less time in proximity with their pairmate upon reunion following a long-separation challenge. During the short-separation challenge, marmosets engaged in more social gaze when separated with an opposite-sex stranger, but not when separated with their mate. Furthermore, marmosets that received the most social gaze from opposite-sex strangers spent the most time in proximity with their long-term mate upon reunion. We also showed that marmosets treated with an OT agonist received increased levels of gaze from opposite-sex strangers, but not from their mate. Overall, these results suggest that marmosets are sensitive to the nature of the social interactions during separation, and subsequently alter their expression of affiliation upon reunion with their long-term mate. These findings further implicate oxytocin as a bond-enhancing molecule that regulates the reestablishment of normative levels of affiliation with a mate following separation, and add to the emerging literature that suggests the OT system underlies critical behavioral processes that contribute to the preservation of long-lasting social bonds.

Keywords

899; L-368; oxytocin; pairbond; Pro⁸-OT; social gaze

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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1 | INTRODUCTION

Given the centrality, complexity, and life-time continuity of social relationships in primates (Platt, Seyfarth, & Cheney, 2016; Silk, Cheney, & Seyfarth, 2013), it should come as no surprise that separation from significant social partners produces a host of neurobiological and behavioral perturbations during the period of separation (French & Carp, 2016; Meyer & Hamel, 2014). Affiliative and prosocial interactions with social partners following separation are critical for the reestablishment of normative relationship dynamics (Fernandez-Duque, Mason, & Mendoza, 1997; Sheperd & French, 1999; Taylor, Mustoe, Hochfelder, & French, 2015). Further, social interactions at reunion serve an important recovery function for behavioral markers of distress during separation, and facilitate the restoration of neurobiological function. The neuropeptide hormone oxytocin (OT) has been implicated in many diverse social processes (Caldwell, 2017; Caldwell & Albers, 2015; Rilling & Young, 2014), including the regulation of the quality of social relationships (Feldman, 2017; Ziegler & Crockford, 2017). Further, OT is linked to social processes that buffer stress responses and underlie the salubrious effects of affiliation on adverse outcomes associated with elevated stress responses (Bosch & Young, 2017; Gobrogge & Wang, 2015; Smith & Wang, 2012). In light of this, it is reasonable to assume that OT modulates social interactions that serve to reestablish social and physical proximity upon reunion after a period of social separation or isolation.

The biparental and pair-living marmoset monkey (*Callithrix* spp.) lives in a complex, nested set of social relationships that includes infants and caregivers (including mothers, fathers, and older siblings), juvenile and adult-aged offspring residing in the family group, and especially between heterosexual breeding adults in the group. The consequences of both short- and long-term separation within these classes of relationships have been studied in marmosets, including parent-offspring separation (Birnie, Taylor, Cavanaugh, & French, 2013; Dettling, Feldon, & Pryce, 2002; French et al., 2012), juveniles isolated from family groups (Cinini et al., 2014; French et al., 2012; Mustoe, Taylor, Birnie, Huffman, & French, 2014; Taylor, Mustoe, & French, 2014), and in long-term heterosexual or same-sex adult partners (Cavanaugh, Carp, Rock, & French, 2016; Cavanaugh, Huffman, Harnisch, & French, 2015; Duarte, Maior, & Barros, 2017; Galvão-Coelho, Silva, & De Sousa, 2012; Norcross & Newman, 1999; Rukstalis & French, 2005; Smith, McGreer-Whitworth, & French, 1998). In all cases, separation leads to elevated levels of glucocorticoid production and multiple behavioral changes associated with increased distress. These studies also reveal that higher rates of affiliative behavior during reunion with social partners is associated with a quicker return to normative glucocorticoid levels and a reduction in distress-related behavioral patterns. There is burgeoning evidence that the OT system regulates the anxiolytic and stress-reducing effects of social buffering in primates (Cavanaugh et al., 2016; Crockford, Deschner, & Wittig, 2017; Parker, Buckmaster, Schatzberg, & Lyons, 2005); yet we know relatively little about neuroendocrine regulation of received and initiated affiliative behavior during reunion.

In marmosets, OT is an important modulator of social behavior across several social realms, as revealed through studies that correlate endogenous measures of OT with sociality, and by

studies that pharmacologically manipulate the OT system. These social realms include offspring care and food provisioning (Finkenwirth, Martins, Deschner, & Burkart, 2016; Saito & Nakamura, 2011; Taylor & French, 2015), social relationships between pair-bonded males and females (Cavanaugh, Mustoe, Taylor, & French, 2014; Finkenwirth, van Schaik, Ziegler, & Burkart, 2015; Smith, Ågmo, Birnie, & French, 2010; Snowdon et al., 2010), and cooperation and altruism in food sharing tasks (Mustoe, Cavanaugh, Harnisch, Thompson, & French, 2015). Although the role of OT in the regulation of social behavior upon reunion in marmosets has not yet been addressed, data from the pairliving and socially monogamous titi monkey (*Callicebus moloch*) suggests that changes in endogenous OT release may facilitate reunion in this species, particularly for prolonged social separations. In male titi monkeys, CSF OT is elevated during both prolonged (48 hr) and long-term (2–3 weeks) separations from pairmates (Hinde et al., 2016). Reunion with their pairmate, but not exposure to an opposite-sex stranger, following the separations stimulated coordinated release of both CSF and plasma OT, suggesting that the OT response is induced specifically by the long-term partner, and that the elevated OT response may facilitate social behavior that is specific to long-term partners in monogamous primates. Like other measures of sociality, the OT system appears to be involved in reunion with a long-term pairmate following a period of separation.

In this paper, we explore the role of the OT system in regulating initiated and received affiliative behavior during reunion following short and long social separation and social isolation in adult heterosexual pairs of marmosets via pharmacological manipulation of the OT system. Further, we tested OT ligand-specificity in modulating social behavior by administering two unique OT ligands: Leu⁸-OT, the consensus mammalian ligand variant; Pro⁸-OT, the ligand variant present in marmosets (French, Taylor, Mustoe, & Cavanaugh, 2016; Mustoe, Taylor, & French, 2018; Ren et al., 2015). We monitored broad patterns of affiliative behavior immediately upon reunion, including initiated and received grooming behavior, and social proximity to the partner. To the extent that OT modulates reunion-related affiliative interactions, we predicted higher rates of grooming and social proximity among pairmates after OT agonist treatment, and reduced rates of these interactions upon reunion with OT antagonist treatment (OTA). Furthermore, if structural changes in the OT ligand alter OT signaling properties, then treating marmosets with Pro⁸-OT, but not Leu⁸-OT, should increase the expression of affiliation with a mate upon reunion. We also explored whether reunion behavior differed following long and short-term isolation, long- and short-term separations with a mate, and finally short-term separations with an opposite-sex stranger. By comparing OT treatments across multiple social contexts and durations of a separation challenge, we can evaluate whether the OT system regulates reunion behavior regardless of the context or duration of separation, or whether the OT system modulates reunion behavior differentially as a property of the nature and duration of separation.

2 | GENERAL METHOD

These experiments examined the extent that the OT-system regulates the expression of affiliative behavior with a long-term pairmate upon reunion following a long-separation challenge (Experiment 1), as well as the extent that the OT-system alters behavioral responses during a short-separation challenge and the expression of affiliative behavior with

a long-term pairmate upon reunion following a short-separation challenge (Experiment 2). The separation challenges varied in both social context (isolation vs. separation) and duration (long vs. short). During isolation conditions, marmosets were not in the presence of others, and, therefore, were unable to engage in social interactions. During separation conditions, marmosets had visual, acoustic, and olfactory, but not physical access to a social partner. The long-separation challenge included social-context conditions of social isolation and mate separation, while the short-separation challenge included social-context conditions of social isolation, mate separation, and opposite-sex stranger separation. The social-context manipulations for both the long-separation (7 hr) and the short-separation (15 min) challenges were preceded by a 30-min social isolation for drug uptake (Figure 1). The data for these experiments were collected during earlier studies (Cavanaugh et al., 2016; Mustoe et al., 2015; Mustoe, Harnisch, Hochfelder, Cavanaugh, & French, 2016) and have not been previously published.

2.1 | Subjects

These experiments were conducted with marmosets (*Callithrix* spp.), a highly social, cooperatively-breeding New World primate that readily form and maintain long-term, male-female relationships (Digby, 1995; Schaffner, Shepherd, Santos, & French, 1995) and experience a pronounced stress response to social disruption (Rukstalis & French, 2005). In marmosets, a single nucleotide substitution in the coding region of the *OT* gene leads to a unique OT ligand (Pro⁸-OT), with distinct structural and physicochemical properties from consensus mammalian OT (Leu⁸-OT; Lee et al., 2011; Ren et al., 2015; Vargas-Pinilla et al., 2015). Furthermore, this ligand variation is associated with significant changes in the marmoset oxytocin receptor (OTR; Ren et al., 2015) and social phenotype (Cavanaugh et al., 2014, 2015; Mustoe et al., 2015). Marmosets are housed at the Callitrichid Research Center (CRC) at the University of Nebraska at Omaha in large indoor wire-mesh enclosures (1.0 × 2.5 × 2.0 m) that are equipped with a sleeping hammock, natural branches for climbing and various enrichment materials. Visual access is restricted between home enclosures, but auditory and olfactory cues are not. Colony rooms at the CRC are maintained on a 12 hr: 12 hr light: dark cycle and at a temperature range between 19 and 22 °C. For all dietary and husbandry protocols please refer to (Schaffner et al., 1995). All procedures were approved by the University of Nebraska at Omaha/University of Nebraska Medical Center Institutional Animal Care and Use Committee (#12-099-12; #13-048-07) and adhered to the ethical standards endorsed by the American Society of Primatologists.

2.2 | Oxytocin treatments

Marmosets received four drug treatments ([1] Pro⁸-OT agonist; [2] Leu⁸-OT agonist; [3] OTA; [4] Saline control) at doses determined by previous primate literature (Boccia, Goursaud, Bachevalier, Anderson, & Pedersen, 2007; Cavanaugh et al., 2014; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Parker et al., 2005; Smith et al., 2010). At 90 min prior to behavioral testing, marmosets received the OTA (20 mg/kg L368,899@; provided by Dr. Peter Williams, Merck & Co., Inc.) via oral administration in a preferred food item in their home enclosure. Marmosets received a saline-treated food item during Pro⁸-OT, Leu⁸-OT, and saline intranasal treatment conditions. L368,899@ is readily absorbed by the bloodstream after passage through the digestive system (Thompson et al.,

1997), reaches peak concentration in the cerebrospinal fluid (CSF) 90 min after administration, is maintained at 50% peak concentration 4+ hr after administration, and accumulates in areas of limbic system (Boccia et al., 2007).

At 30 min prior to behavioral testing marmosets were administered 150 µg/kg (50 µg/100µl saline solution) of two separate OT agonists (i.e., Pro⁸-OT, synthesized by Anaspec Corp, CA; Leu⁸-OT; provided by Maurice Manning, University of Toledo), or saline control via intranasal administration during a brief (~3 min) manual restraint using a 100-µl Eppendorf pipette to administer 50 µl of solution to each nostril drop-wise. Marmosets also received intranasal saline during the OTA treatment condition. Peptides administered intranasally are quickly absorbed into the bloodstream via the nasal passage (Pires, Fortuna, Alves, & Falcão, 2009), and some fraction of the peptides 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 & Feifel, 2013; Quintana, Alvares, Hickie, & Guastella, 2015). Intranasal neuropeptides are transported to the CNS and accumulate in the CSF in humans (Striepens et al., 2013) and macaques (Dal Monte, Noble, Turchi, Cummins, & Averbeck, 2014). In rats and mice, OT levels were increased in microdialysates from the hippocampus and amygdala, and in plasma, 30–60 min after intranasal administration (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013). Peripheral levels of OT after intranasal administration peak at 60 min and persist for up to 7 hr in humans (van IJzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012). Following intranasal administration, marmosets were transferred to a transport enclosure (0.3 × 0.3 × 0.3 m) and remained isolated in a quiet room some distance from the colony room that contained the pair's home enclosure for 30 min, after which behavioral testing commenced.

3 | EXPERIMENT 1

3.1 | Behavioral paradigm

Ten nulliparous (five male and five female) adult marmosets (*C. jacchus*) were utilized in the long-separation challenge. Animals were 4.3 ± 0.2 (mean $\pm$ SEM) years of age and had cohabitated with the same partner for 15 months by the start of the experiment. Marmosets were exposed to a long-separation challenge (7 hr) over a series of eight counterbalanced conditions. Marmosets experienced two context conditions ([1] social isolation; [2] mate separation) and four drug-treatment conditions ([1] Pro⁸-OT agonist; [2] Leu⁸-OT agonist; [3] OTA; [4] saline control). Each member of the male-female pair was treated separately. Following the 30-min drug-uptake period, the subject was transferred to a larger enclosure (0.6 × 0.6 × 0.6 m) with access to food and water at 0900 hr and remained in this environment until 1600 hr. For each drug-treatment condition, the subject experienced the long-separation challenge once with their pairmate present but physically inaccessible (mate separation), and once without their pairmate present (social isolation); the distance between adjacent enclosures was 0.3 m. During mate separation trials, the subject had visual, acoustic, and olfactory, but not physical access to their untreated mate. During social isolation trials, an empty enclosure was present next to the subject and the untreated pairmate remained in the home enclosure. There was a 2–4 week washout period between

consecutive conditions. For additional information on the methodology please refer to Cavanaugh et al. (2016).

Following the long-separation challenge (0900–1600 hr), marmosets were reunited with their pairmate in their home enclosure. During this reunion, social interactions between pairmates were observed and scored by treatment-blind observers for 15 min. Social interactions between pairmates were also observed and scored for 15 min at the same time of day the day prior to (baseline), and the day after (post), the long-separation challenge for each of the eight conditions to obtain typical measures of pair sociality. Marmosets did not receive any drug treatments and were not removed from their home enclosure on the day of the baseline and post observations. Refer to Table 1 for a description of the behaviors examined. All observers were trained to a reliability of $\kappa > 0.90$. All focal animal observations were recorded using Stopwatch+ software (Emory University).

3.2 | Data analysis

We evaluated the effects of drug treatment and social context on behavioral measures exhibited upon reunion with a pairmate following the long-separation challenge using several-mixed model ANOVAs, with drug treatment ([1] Pro⁸-OT agonist; [2] Leu⁸-OT agonist; [3] OTA; [4] saline control) and social context ([1] social isolation; [2] mate separation), as within-subject factors, and sex as a between-subject factor ($4 \times 2 \times 2$). Additional analyses were conducted on drug-treatment, social context, and observation ([1] baseline; [2] reunion; [3] post) ($4 \times 2 \times 3$) to determine the extent that the long-separation challenge altered normative pair interactions. If main effects or interactions were significant, post-hoc comparisons were made using least squared difference. All alpha levels were set at $p < 0.05$.

4 | RESULTS

Exposure to a long-separation challenge substantially increased sociality with a pairmate upon reunion, relative to baseline and post levels of sociality, collapsed across drug treatment and the context of the separation (Supplemental Table S1). The interaction between drug treatment and the context of the separation did not impact several measures of sociality with a pairmate upon reunion after separation (Supplemental Table S2). Sex of the marmoset did not significantly interact with drug treatment or social context to impact any of the measures of social behavior during reunion following the separation challenge. However, drug treatment and the context of the separation independently impacted several measures of affiliative behavior during reunion.

4.1 | Pair-directed proximity and huddling behavior during reunion

Drug treatment and the context of the long separation independently influenced joint affiliation between long-term pairmates during reunion. Drug treatment significantly altered the total duration of time marmosets spent in proximity with their pairmate ($F_{3,27} = 2.97$, $p = 0.05$, $\eta^2 = 0.25$; Figure 2a) and the mean duration of time per proximity bout ($F_{3,27} = 3.05$, $p = 0.046$, $\eta^2 = 0.25$) upon reunion. Marmosets that received an OTA prior to the separation challenge spent significantly less time in proximity with their pairmate upon

reunion, relative to a saline control ($p < 0.05$). Treatment with either Pro⁸-OT or Leu⁸-OT did not significantly impact the duration of time marmosets spent in proximity with their pairmate upon reunion, relative to a saline control. Drug treatment did not affect the total duration of time marmosets spent huddling with their pairmate during reunion ($F[3,27] = 0.70$, $p = 0.56$, $\eta^2 = 0.07$) or the mean duration of time per huddling encounter ($F[3,27] = 0.56$, $p = 0.65$, $\eta^2 = 0.06$) upon reunion.

The context of the separation independently influenced several measures of pair behavior upon reunion with a mate. Marmoset pairs spent moderately more time in proximity upon reunion ($F[1,9] = 3.44$, $p = 0.09$, $\eta^2 = 0.28$; Figure 2b), but not more time per proximity bout ($F[1,9] = 0.18$, $p = 0.68$, $\eta^2 = 0.02$), following mate separation than following social isolation. The context of the separation did not significantly impact the total duration of time marmosets spent huddling with their pairmate ($F[1,9] = 0.61$, $p = 0.45$, $\eta^2 = 0.06$) or the mean duration of time per huddling encounter ($F[1,9] = 0.74$, $p = 0.41$, $\eta^2 = 0.08$), upon reunion.

4.2 | Subject-initiated proximity and grooming behavior during reunion

The context of the long separation, but not drug treatment, influenced subject-initiated affiliation with a long-term pairmate during reunion. Drug treatment did not significantly affect the frequency ($F[3,27] = 0.04$, $p = 0.99$, $\eta^2 = 0.01$) or latency ($F[3,27] = 0.23$, $p = 0.87$, $\eta^2 = 0.03$; Figure 2c) that marmosets approached their untreated pairmate during reunion, nor did it affect the total duration of time ($F[3,27] = 0.29$, $p = 0.84$, $\eta^2 = 0.03$; Figure 2e) or the mean duration of time marmosets spent allogrooming their pairmate per bout ($F[3,27] = 0.49$, $p = 0.69$, $\eta^2 = 0.05$) during reunion. However, the context of the separation independently influenced subject-initiated proximity behavior. Marmosets approached their pairmate significantly more frequently ($F[1,9] = 5.63$, $p = 0.04$, $\eta^2 = 0.39$) and significantly more quickly ($F[1,9] = 8.93$, $p = 0.015$, $\eta^2 = 0.50$; Figure 2b) following mate separation than following social isolation. The context of the separation did not significantly impact the total duration of time ($F[1,9] = 2.94$, $p = 0.12$, $\eta^2 = 0.25$; Figure 2f), or the mean duration of time marmosets spent allogrooming their pairmate per bout ($F[1,9] = 2.70$, $p = 0.14$, $\eta^2 = 0.23$), upon reunion.

4.3 | Subject-received proximity and grooming behavior during reunion

Drug treatment and the context of the long separation independently influenced received affiliation from an untreated pairmate during reunion. Drug treatment significantly altered the total duration of time marmosets were groomed by their untreated pairmate ($F[3,27] = 3.08$, $p = 0.044$, $\eta^2 = 0.26$; Figure 2i), and moderately altered the mean duration of time marmosets were groomed by their untreated pairmate per bout ($F[3,27] = 2.34$, $p = 0.09$, $\eta^2 = 0.21$), upon reunion following the long-separation challenge. Marmosets administered an OTA or Leu⁸-OT prior to the separation challenge received less grooming by their untreated pairmate upon reunion, relative to a saline control ($p < 0.05$). Treatment with Pro⁸-OT did not significantly impact the duration of time that marmosets received grooming from their pairmate upon reunion, relative to a saline control. Drug treatment did not significantly affect the frequency that marmosets solicited grooming from their untreated pairmate ($F[3,27] = 1.47$, $p = 0.25$, $\eta^2 = 0.14$) during reunion. Drug treatment also did not

significantly affect the frequency ($F[3,27] = 1.12, p = 0.36, \eta^2 = 0.11$) or latency ($F[3,27] = 1.45, p = 0.25, \eta^2 = 0.14$; Figure 2g) that marmosets were approached by their untreated pairmate during reunion.

The context of the separation independently influenced received grooming behavior, but not received proximity behavior, upon reunion with a mate. Marmosets received significantly longer bouts of grooming from their untreated pairmate ($F[1,9] = 6.96, p = 0.03, \eta^2 = 0.44$), and were groomed for a longer total duration ($F[1,9] = 4.30, p = 0.06, \eta^2 = 0.32$; Figure 2j) following social isolation than following mate separation. Social context did not significantly affect the frequency that marmosets solicited grooming from their pairmate ($F[1,9] = 0.03, p = 0.88, \eta^2 = 0.01$). Marmosets were not approached more often ($F[1,9] = 1.65, p = 0.231, \eta^2 = 0.16$) or more quickly ($F[1,9] = 1.66, p = 0.229, \eta^2 = 0.16$; Figure 2HD) by their pairmate across context conditions.

5 | DISCUSSION

The expression of affiliation with a pairmate following a period of social isolation or separation is essential, not only to buffer against detrimental effects of exposure to social stressors, but also to reestablish social and physical proximity and cultivate attachment with a long-term mate. Here, we demonstrated that pharmacological manipulation of the OT system impacted the expression of some measures of affiliation with a long-term pairmate following a long-separation challenge. OTA treatment had the most pronounced effects on the expression of affiliation with a mate following a long-separation challenge. Specifically, marmosets treated with an OTA engaged in less social proximity and received less grooming behavior from their untreated pairmate following a long separation.

The OTA-induced reduction in pair proximity and received grooming behavior upon reunion effect in the current study supports and extends previous research that showed treatment with an OTA increases the magnitude of the physiological stress response and reduces the time spent in proximity to an adjacent enclosure that houses the subjects' long-term mate (Cavanaugh et al., 2016). While we expected that untreated pairmates would be sensitive to their OTA-treated partner's increased stress levels and responsively increase their expression of affiliation to comfort their OTA-treated partner, OTA-treated marmosets actually received *less* grooming behavior from their untreated pairmate during reunion. We found no effect of OT treatment on the frequency that marmosets solicited grooming from their untreated pairmate, suggesting that antagonism of the OTR may modify more subtle behavioral features that render them less socially attractive or reduce signals that indicate behavioral distress. Alternatively, it may be that marmosets received less grooming behavior as a consequence of the reduction in joint proximity within pairs as a result of OTA treatment. Since the OTA (L368,899®) is maintained in the CSF at 50% peak concentration 4+ hr after administration (Boccia et al., 2007), one possible limitation of the effects of OTA in the current study is that pharmacological properties of the OTA may not have persisted following the 7 hr separation challenge. However, the OTA had pronounced effects on both HPA-axis activity and the expression of social behavior during the 7 hr separation challenge (Cavanaugh et al., 2016), and this altered experience may have carried over into reunion with a long-term pairmate.

While OTA treatment markedly decreased initiated proximity during the long-separation challenge (Cavanaugh et al., 2016), as well as joint and received affiliation during reunion (current study), OT agonist treatment did not alter the expression of initiated or received affiliation during separation or reunion. While administration of intranasal OT has been shown to modulate social behavior in marmoset pairs (Cavanaugh et al., 2014, 2015; Mustoe et al., 2015; Smith et al., 2010), there may be differential thresholds for alteration of social behavior by OTR activation across social contexts and across measures of social behavior. Here, our findings suggest that the endogenous OT system may be an important regulator of initiated proximity with a mate during periods of long separation and an important regulator of *received* affiliation during reunion, likely by modifying behavioral properties that signal their pairmate that they require social attention and affiliation.

The social context of the long-separation challenge also had pronounced effects on the expression of affiliation upon reunion with a long-term mate. Marmosets that were isolated during the long-separation challenge (7 hr) approached their pairmate more often and more quickly, and were groomed more often, during reunion compared to when they were separated with their mate. This finding is consistent with previous studies, as marmosets have been shown to spend more time in proximity with their mate upon reunion following prolonged isolation (48 hr) than following prolonged separation with their mate (48 hr; Smith et al., 1998). In titi monkeys, overall levels of grooming behavior and several measures of arousal, but not measures proximity and physical contact, upon reunion with a mate were increased following a prolonged isolation (5 days) compared to a short isolation (<2 hrs; Fernandez-Duque et al., 1997). These results suggest that marmosets and titi monkeys are sensitive to the magnitude of social isolation and likely utilize reunion behavior to buffer their stress levels and reestablish normative levels of sociality with their mate.

Sociality upon reunion serves an important role in regulating the return to baseline biobehavioral function in marmosets. Following a short isolation (1 hr), marmosets showed *increased* grooming and proximity upon reunion with their mate, and locomotor behavior (a behavioral correlate of distress) was reduced relative to isolation levels (Shepherd & French, 1999). In the context of short-term isolation of adult age offspring from the family group, levels of initiated grooming behavior upon reunion were substantially increased compared to undisturbed social interactions. Moreover, higher levels of initiated grooming behavior, but not received grooming from family members, was associated with better regulation (i.e., return to baseline) of the stress response (Taylor et al., 2015). This suggests that in adult aged offspring the active initiation of affiliation upon reunion, but not the passive receipt of grooming from family members, is important for biobehavioral regulation upon reunion. Moreover, these findings indicate that affiliation with social partners following separation directly impacts stress recovery; the results of the current study suggest that the OT system regulates both the initiation and receipt of affiliative behavior during reunion, likely producing the salubrious effects of social buffering and reinforcing the long-term social bond.

6 | EXPERIMENT 2

6.1 | Behavioral paradigm

Nine nulliparous (five male and four female) adult marmosets (six *C. penicillata*; three *C. jacchus*) were utilized in the short-separation challenge. Animals were 4.5 ± 1.5 years of age at the onset of experiments and had cohabitated with the same partner for a minimum of two months prior to the start of the experiment. Marmosets were exposed to a short-separation challenge over a series of twelve counterbalanced conditions. Marmosets experienced three context conditions ([1] social isolation; [2] mate separation; [3] opposite-sex stranger separation) and four drug-treatment conditions ([1] Pro⁸-OT agonist; [2] Leu⁸-OT agonist; [3] OTA; [4] saline control). Each member of a male–female pair was treated and tested separately. For each drug-treatment condition, the subject experienced the short-separation challenge once with their pairmate present but physically inaccessible (mate separation), once with an opposite-sex stranger present but physically inaccessible (opposite-sex stranger separation), and once without their pairmate or a stranger present (social isolation); the distance between adjacent enclosures was 0.3 m. During the separation trials, the subject had visual, acoustic, and olfactory, but not physical access to a social partner (untreated pairmate or untreated opposite-sex stranger). During these trials, marmosets were able to engage in social interactions including social approach, social gaze, vocalizations, and sociosexual or antagonistic displays, in addition to the experimental task (i.e., food provisioning). During social isolation trials, an empty enclosure was present next to the subject and the untreated social partner remained in the home enclosure.

During the short-separation challenge, marmosets participated in a series of prosocial-choice tasks that were designed to measure the influence of the OT system on “altruistic food sharing” (Mustoe et al., 2015) and inequity aversion (Mustoe et al., 2016). In summary, the tasks consisted of trained marmosets participating in a series of trials where they were able to make social decisions to provision food rewards to a partner that was either the donor marmoset’s long-term mate or an opposite-sex stranger. Thus the short-separation challenge consisted of active social interactions where individuals provisioned food rewards using a modified tray-pulling paradigm described in (Mustoe et al., 2015, 2016), similar to a paradigm used previously (Burkart, Fehr, Efferson, & van Schaik, 2007).

The short-separation challenge lasted ~15 min (following the 30-min drug-uptake period) and was video recorded and scored for social gaze and proximity between the donor and recipient by treatment-blinded observers. Immediately following the short-separation challenge, subjects were reunited with their pairmate in their home enclosure and treatment-blinded observers live scored behavior for 10 min (altruism study) or 5 min (inequity study). Refer to Table 1 for a description of the behaviors examined. All observers were trained to reliability of $\kappa > 0.90$. All focal animal observations were recorded using Stopwatch + software (Emory University).

6.2 | Data analysis

We evaluated the effects of drug treatment and social context on behavioral measures exhibited upon reunion with a pairmate following the short-separation challenge using

repeated-measures ANOVAs, with drug treatment ([1] Pro⁸-OT agonist; [2] Leu⁸-OT agonist; [3] OTA; [4] saline control) and the context of the challenge ([1] social isolation; [2] mate separation; [3] opposite-sex stranger separation) as within-subject factors (4 × 3). One male–female pair was omitted from the reunion grooming analyses because they never exhibited grooming behavior across any observations. If main effects or interactions were significant, post-hoc comparisons were made using least squared difference. All alpha levels were set at $p < 0.05$.

To further evaluate whether social behavior during the short-separation challenge modulates the relationship between drug treatment, the context and the separation, and behavior upon reunion we conducted two generalized estimating equations (GEE) models using repeated measures across individuals with mate and stranger (social), drug treatment, with one model covarying gaze initiated and gaze received, and another covarying proximity initiated and proximity received. These models provided estimates of OT and social factors on proximity duration during reunion, while specifically accounting for variation in initiated and received social behavior during the short separations. The GEE analyses are well-suited for repeated measure factorial analyses with small sample sizes, and are also effective at accounting for correlations within subjects, across factors, and within-subject dependency on the outcome measures (Muth et al., 2015). Measures across individuals were balanced and order effects were counter-balanced across separations and therefore time or order effects were not analyzed. Independent correlation matrices provided the best model fits and were used for both analyses; though, notably, there were very few significant differences in model effects using exchangeable correlation matrices, which have been utilized previously (Hinde et al., 2016). GEE was preferred over GLMM models due to issues with convergence on the measure of proximity during separation.

We sought to statistically evaluate the assumption of whether social behaviors during short-term separations were associated with reunion proximity by using GEE models to account for multivariate factors including OT treatment and the social context of the separation (i.e., whether separated near a pairmate or an opposite sex stranger). We measured two primary social behavior domains during the social separation (e.g., social gaze and social proximity). Each of these social behaviors can be classified in two ways: (i) behavior *initiated* by the OT-treated marmoset and (ii) behavior *received* by or directed toward the OT-treated marmoset from the adjacent pairmate or opposite-sex stranger. The GEE model evaluated whether OT treatment and social context were associated with reunion proximity when covarying for social behavior exhibited during the separation (e.g., initiated and received social gaze).

Given the nature of the GEE models including excessive multiple comparisons for hypothesis testing, we have taken a conservative approach to interpreting individual model effects and parameter estimates in these models. First we used correlation analyses (e.g., Spearman correlation) to evaluate whether there was a significant relationship between behavior during the separation (social gaze or social proximity) and reunion proximity. For behavior(s) that were significantly associated with reunion proximity (i.e., social gaze), we further analyzed the relationship between reunion proximity as predicted by OT treatments, social context, and variation in social gaze using GEE models. The model effects can be

viewed as similar to an omnibus test as to whether an individual factor (e.g., OT treatment or social context) or factor interaction (OT treatment by social context) significantly contributes to explaining variability in reunion proximity. Only in cases of significant model effects, then, do we interpret any potential significant parameter estimates (levels within an individual factor and/or factor interaction) and posthoc comparisons. Posthoc comparisons were performed only on significant model effects/parameter estimates based on GEE estimated means (i.e., means covaried with social gaze).

7 | RESULTS

7.1 | Pair-directed proximity, and initiated and received grooming behavior during reunion

Time spent in proximity with a pairmate during reunion was not affected by drug treatment ($F[3,24] = 0.53, p = 0.67, \eta^2 = 0.06$), but was significantly influenced by the context of the short-separation challenge ($F[2,16] = 7.60, p = 0.005, \eta^2 = 0.49$). Specifically, marmosets spent significantly more time in proximity with their mate during reunion following separation with an opposite-sex stranger compared to when they were separated with their mate ($t[8] = 3.20, p = 0.01$) or socially isolated ($t[8] = 2.90, p = 0.02$). There was no significant interaction between drug treatment and the context of the separation on proximity duration ($F[6,48] = 0.72, p = 0.64, \eta^2 = 0.08$; Supplemental Table S2). The mean duration of time per proximity encounter did not differ by drug treatment ($F[3,24] = 0.51, p = 0.68, \eta^2 = 0.06$), but increased following separation with an opposite-sex stranger ($F[2,16] = 3.45, p = 0.057, \eta^2 = 0.30$), compared to when separated with their mate ($t[8] = 2.36, p = 0.046$) or socially isolated ($t[8] = 2.26, p = 0.054$).

Neither drug treatment ($F[3,18] = 1.92, p = 0.16, \eta^2 = 0.24$) nor the context of the separation ($F[2,12] = 0.04, p = 0.96, \eta^2 = 0.01$; Figure 5c) significantly affected the duration that marmosets groomed their untreated pairmate during reunion, and there was no significant interaction between drug treatment and the context of the separation ($F[6,36] = 1.19, p = 0.37, \eta^2 = 0.16$; Supplemental Table S2). Received grooming from the untreated pairmate during reunion did not differ based on drug treatment ($F[3,18] = 2.18, p = 0.13, \eta^2 = 0.27$) or the context of the separation ($F[2,12] = 0.09, p = 0.92, \eta^2 = 0.01$), and there was no significant interaction ($F[6, 36] = 1.18, p = 0.34, \eta^2 = 0.16$; Supplemental Table S2).

7.2 | Prediction of reunion behavior from social gaze and proximity during separation

Experiencing a short-separation challenge with an opposite-sex stranger was associated with enhanced proximity duration with a mate upon reunion. Specifically, received gaze from an opposite-sex stranger, but not received gaze from their long-term mate, was significantly correlated with time spent in proximity with their mate upon reunion (Stranger separation: $\rho[7] = 0.73, P = 0.025$; Mate separation: $\rho[7] = 0.23, p = 0.55$; Figure 3). Initiated gaze, as well as initiated and received proximity, were not significantly correlated with time spent in proximity with their mate upon reunion ($\rho_s(7) = 0.44, p_s > 0.05$).

Using GEE, we found that a number of factors were significantly associated with the time spent in proximity with their mate upon reunion including gaze directed toward OT-treated marmosets ($\theta[1] = 16.72, p < 0.001$), which partner marmosets were separated with ($\theta[1] =$

8.63, $P = 0.003$), and which OT treatment marmosets received ($\theta[3] = 8.51$, $p = 0.04$; Supplemental Table S3). Specifically, separation with an opposite-sex stranger was associated with increased time spent in proximity with their long-term mate during reunion ($B = 38.43$, $p = 0.008$), and Pro⁸-OT treatment was associated with a reduction in time spent in proximity with a mate during reunion ($B = -37.15$, $p = 0.01$).

We also found that a number of factors significantly interacted to predict proximity with a mate upon reunion. The association between OT treatments and reunion proximity was significantly moderated by social gaze for both initiated gaze ($\theta[3] = 50.12$, $p < 0.01$) and received gaze ($\theta[3] = 11.00$, $p = 0.01$), and the association between OT treatments and reunion proximity was also significantly moderated by who the OT-treated marmoset was separated with ($\theta[3] = 25.68$, $p < 0.001$). The latter effect is particularly important because it suggests that the effect of OT on reunion behavior depends on whom marmosets were separated with even when controlling for the social behavior during the separation. Specifically, when controlling for the amount of social gaze during the separation, saline-treated marmosets separated with an opposite-sex stranger spent significantly more time in proximity with a pairmate upon reunion relative to saline-treated marmosets separated with their pairmate ($p = 0.02$). Moreover, marmosets treated with Pro⁸-OT who were separated with an opposite-sex stranger showed a significant reduction in their reunion proximity compared to saline-treated marmosets separated with a stranger ($p = 0.03$; Figure 4), while there were no significant OT effects on reunion proximity when separated with their mate.

7.3 | Initiated and received social gaze and social proximity during separation

Marmosets' social gaze and social proximity during the short-separation challenge was an important component of predicting time spent in proximity with a long-term mate upon reunion. Consequently, we evaluated how drug treatment and the context of the separation influenced sociality during the short-separation challenge. The duration of marmosets' social gaze was significantly influenced by who they were separated with ($F[2,16] = 22.14$, $p < 0.001$, $\eta^2 = 0.74$), but not the OT treatment they received ($F[3,24] = 0.48$, $p = 0.70$, $\eta^2 = 0.06$). Specifically, marmosets gazed significantly more at opposite-sex strangers during separation than they did at their mate ($t[8] = 4.40$, $p = 0.002$; Figure 5d); there was no significant interaction between the context of the separation and drug treatment ($F[6,48] = 0.59$, $p = 0.74$, $\eta^2 = 0.07$; Figure 5c).

The duration that marmosets received gaze during separation was significantly influenced by who they were separated with ($F[1,8] = 11.95$, $p = 0.009$, $\eta^2 = 0.60$), and by the OT treatment they received ($F[3,24] = 3.20$, $p = 0.04$, $\eta^2 = 0.29$). Additionally, there was a significant interaction between the context of the separation and drug treatment ($F[3,24] = 6.08$, $p = 0.003$, $\eta^2 = 0.43$; Figure 5a). Marmosets received significantly more gaze from an untreated opposite-sex stranger than by their untreated mate ($t[8] = 3.50$, $p = 0.008$; Figure 5b), and marmosets received more gaze from an untreated opposite-sex stranger when they were treated with Pro⁸-OT compared to Leu⁸-OT ($t[8] = 3.04$, $p = 0.01$), OTA ($t[8] = 2.94$, $p = 0.01$), and marginally more compared to saline ($t[8] = 2.14$, $p = 0.06$). Drug treatment did not alter the duration of time that their untreated pairmate gazed at them during separation.

Proximity between marmosets and others during the short-separation challenge did not differ based on drug treatment ($F(3,24) = 1.93$, $p = 0.15$, $\eta^2 = 0.19$). However, proximity between marmosets and others significantly differed based on who they were separated with ($F(1,8) = 1.98$, $p = 0.20$, $\eta^2 = 0.20$), as marmosets spent more time in close proximity to an opposite-sex stranger than to their mate. Conversely, proximity initiated from others did not differ based on drug treatment ($F(3,24) = 0.97$, $p = 0.42$, $\eta^2 = 0.11$) or whether they were a mate or a stranger ($F(1,8) = 1.31$, $p = 0.29$, $\eta^2 = 0.14$), and there was no significant interaction between drug treatment and the context of the separation on initiated ($F(3,24) = 0.13$, $p = 0.94$, $\eta^2 = 0.02$) or received proximity ($F(3,24) = 0.96$, $p = 0.43$, $\eta^2 = 0.11$).

8 | DISCUSSION

While numerous studies have demonstrated that the OT system regulates social motivation and the initiation of social interactions (Caldwell & Albers, 2015), as well as social attention/gaze and socioemotional processing (Chang & Platt, 2014; Domes, Sibold et al., 2013; Domes, Steiner, Porges, & Heinrichs, 2013; Ebitz, Watson, & Platt, 2013; Putnam, Roman, Zimmerman, & Gothard, 2016), less attention has been given to whether augmentation of OT levels alters others' perceptions and behavior *toward* an OT-treated social partner (e.g., social attractiveness). We found that marmosets that received Pro⁸-OT received more gaze from an opposite-sex stranger, but not from their long-term mate, during a short separation. The increased social attention that Pro⁸-OT treated marmosets received from opposite-sex strangers in the current study may have been a cause (or consequence) of marmosets' reduction in food-sharing to opposite-sex strangers during the short-separation challenge (Mustoe et al., 2015). Moreover, we demonstrated that OT-treated marmosets are sensitive to the nature of these social interactions during separation, and subsequently alter their expression of affiliation upon reunion. The social context of the short-separation challenge also impacted the expression of affiliation on reunion behavior. Marmosets that were separated with an opposite sex-stranger (without their pairmate present) spent more time in proximity with their mate upon reunion than when they were separated with their mate or isolated. Moreover, the more social gaze marmosets received from an opposite-sex stranger the more time they spent in proximity with their long-term mate during reunion. These results suggest that marmosets may utilize affiliation during reunion as a way to reinforce the bond with their long-term mate following interactions with an opposite-sex stranger.

One potential explanation for the increased proximity during reunion following separation with an opposite-sex stranger is that marmosets initiated and received different levels of sociality during separation with an opposite-sex stranger than they did during separation with their mate. This difference in the nature of the social interaction during separation may have, in turn, affected expression of affiliative behavior with their mate upon reunion. Marmosets initiated and received more social gaze from an opposite-sex stranger than from their long-term mate during separation. Thus, when the levels of social gaze were held constant, its relative impact on the expression of proximity behavior with a pairmate upon reunion was illuminated. Saline-treated marmosets spent more time in proximity with their mate during reunion after separation with an opposite-sex stranger compared to after separation with their mate, only when accounting for the increased levels of social gaze

during separation trials with an opposite-sex stranger. Moreover, Pro⁸-OT treated marmosets spent less time in proximity with their pairmate following separation with an opposite-sex stranger than when they were treated with a saline control. Since we found that higher levels of received gaze during separation with an opposite-sex stranger, but not during separation with a pairmate, was associated with increased time spent in proximity with a mate upon reunion, the experience of receiving gaze from an opposite-sex stranger carries over into later interactions with a long-term mate. Interacting with an opposite-sex stranger during a separation serves as an important socially-contextual comparison, as expression of affiliation and prosociality is not only differentially sensitive to social context, it is modulated by OT system activity (Cavanaugh et al., 2014; Mustoe et al., 2015). While we do not know to what extent HPA-axis activity differed following these three social contexts, we expect that experiencing short separations with a mate, an opposite-sex stranger, or alone were likely both arousing and rewarding, and minimally aversive, particularly because marmosets were engaged in a series of prosocial-choice tasks. Future studies should continue to investigate this ecologically valid question that has important implications for monogamy, reproductive, and prosociality.

Social gaze is one of the most important tools that primates use to communicate and navigate their social worlds. The ability to dynamically identify and process relevant information about the meaning of a subtle glance or the significance of a lasting stare is a critical skill for thriving in complex social environments. Misinterpretation of these visual clues or failure to appropriately respond can adversely affect individual social interactions and relationship quality. While OTRs are unsurprisingly distributed across the social-decision-making network in primates, the OT system also has a substantial presence within neural circuits that regulate selective attention (French et al., 2016). The overall patterns of receptor distribution in the visual processing areas in primates is consistent with the importance and predominance of visual stimuli in primate social relationships, akin to the central role of olfaction, and the rich expression of OTR in odor-processing networks, in rodents (Freeman & Young, 2016).

Given that the social interactions during separation were dynamic and dyadic (i.e., non-independent), altered expression of received gaze when marmosets were treated with Pro⁸-OT could be the result of behavioral change from either the subject or the opposite-sex stranger. It may be that Pro⁸-OT treated marmosets received longer durations of gaze from an opposite-sex stranger as a response to the decrease in food-sharing that occurred during these trials (Mustoe et al., 2015) (i.e., strangers gaze while awaiting food sharing). Alternatively, Pro⁸-OT treated marmosets may have been more sensitive and reactive to the increased gaze from opposite-sex strangers which resulted in more vigilant and less prosocial responses toward strangers. OT generally promotes the expression of social gaze and attention in rhesus macaques and humans (Bartz, Zaki, Bolger, & Ochsner, 2011; Chang, Barter, Ebitz, Watson, & Platt, 2012; Guastella, Mitchell, & Dadds, 2008; Putnam et al., 2016), but it also attenuates vigilant responses to aversive facial expressions and dominant individuals in macaques (Ebitz et al., 2013; Parr, Modi, Siebert, & Young, 2013). The reproductive and social systems of marmosets and macaques are not analogous and macaques are likely more vigilant-oriented, however, both selectively engage in social vigilance to reduce the occurrence of costly aggressive encounters (Treves, 2000). While we

cannot ascertain the valence of the received gaze from the opposite-sex stranger in the current study, we postulate that it was likely both investigatory and used as a signal of social solicitation. Consequently, valence perception and the resultant behavioral responses during separation, as well as upon reunion with a long-term mate, have important consequences for the integrity of the established bond.

We also found that the dynamic interactions with a social partner during the short-separation challenge carried over and impacted the expression of affiliation with a pairmate during reunion. The manner in which this behavior carried over into reunion appears to be socially- and OT- specific when accounting for the amount of gaze during the social interaction. Overall, these results suggest that OT enhances marmosets' context-specific sensitivity to received gaze from opposites-sex strangers, which subsequently reduces the expression of proximity with their mate upon reunion. While it's not yet clear to what degree OT-system modulation impacts others' perceptions and behavior *toward* an OT-treated social partner, our results suggest that the OT system modifies the expression of prosocial behavior and/or sensitivity and reactivity to increased attention from strangers. This finding that OT-treated marmosets altered their expression of mate-directed affiliation upon reunion after receiving increased social attention from opposite-sex strangers adds to the emerging literature that suggests that the OT system underlies behavioral processes that contribute to the preservation of long-term relationships (French, Cavanaugh, Mustoe, Carp, & Womack, 2017).

9 | GENERAL DISCUSSION

Within long-term social relationships, the expression of affiliative and prosocial behaviors with a partner following a period of separation not only provides vital social support and buffers against detrimental consequences associated with separation, but also reestablishes normative social behavior between social partners. Here, we examined the extent to which the OT-system regulates the expression of affiliative behavior with a long-term pairmate upon reunion following social separation. We demonstrated that the impact of OT-system modulation of reunion behavior largely depends on the context and magnitude of social separation. Specifically, marmosets treated with an OTA engaged in less social proximity and received less grooming behavior from their untreated pairmate following a long separation, but not following a short separation. We showed that marmosets that received the most social gaze from opposite-sex strangers during a short-separation challenge spent the most time in proximity with their long-term mate during reunion. We also found that marmosets treated with Pro⁸-OT received more gaze from opposite-sex strangers, but not from their mate. These results suggest that the OT system impacts reunion behavior in a variety of ways that depend both on the duration of the separation and the presence of their mate during the separation.

While OT-system modulation appreciably altered sociality upon reunion following a long-separation challenge (7 hr), it had minimal impact on sociality upon reunion following a short-separation challenge (15 min). This suggests that OT's role in modulating affiliation with a long-term mate upon reunion is, in large part, contingent on the magnitude of the separation. Our results suggest that the OT-system may be sensitive to separations along a

continuum, such that social interactions upon reunion following longer separations are more sensitive to OT system activity. We found that OT treatments had more prominent effects following a long-separation challenge that was designed to induce biobehavioral distress (Cavanaugh et al., 2016) than following a short-separation challenge that was designed to be minimally stressful (Mustoe et al., 2015, 2016). In male titi monkeys (*Callicebus cupreus*), levels of CSF OT were elevated during both prolonged (48 hr) and long-term (2–3 weeks) separation from a pairmate (Hinde et al., 2016). While CSF OT levels remained elevated during reunion with their pairmate *and* during exposure to opposite-sex strangers, following separation, reunion with their pairmate stimulated coordinated release of both central and peripheral OT. Moreover, males that previously reproduced with their pairmate had lower neural activity in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (central hubs of OT production) during exposure to an opposite-sex stranger, than males that had not reproduced with their pairmate (Hinde et al., 2016). This suggests that oxytocinergic activity underlies reunion behavior differently as a function of experiences in the pair (i.e., raising vs. not raising offspring), and that the OT system is sensitive to the level of reproductive success of an individual's current relationship and implicates fatherhood as bond-enhancing experience. The current study utilized nulliparous male and female marmosets in well-established pairs; thus, it would be useful to assess the impact of previous shared experiences in rearing offspring on the impact of OT manipulations on behavior upon reunion.

Administration of intranasal Pro⁸-OT (marmoset OT ligand), but not Leu⁸-OT (the consensus mammalian variant of OT) significantly impacted the expression of affiliation during the short-separation challenge, which in turn, carried over into interaction with a long-term mate during reunion. Previously, we demonstrated that intranasal Pro⁸-OT exerted more potent effects than intranasal Leu⁸-OT on fidelity-threatening behaviors in marmoset pairs, by decreasing social motivation to interact with an opposite-sex stranger (Cavanaugh et al., 2014; Mustoe et al., 2015). Administration of intranasal Leu⁸-OT has, however, been shown to modulate social behavior in marmoset pairs (Cavanaugh et al., 2015; Smith et al., 2010), as well as father-offspring interactions (Saito & Nakamura, 2011). Due to their distinct structural and physiochemical profiles, Leu⁸-OT and Pro⁸-OT are predicted to have differential binding affinities for the marmoset OTR, differential intracellular signaling cascades, and potentially differential sensitivity of neural circuits that mediate expression of the social phenotype. While Leu⁸-OT and Pro⁸-OT appear to elicit distinct signaling profiles in the human OTR (Parreiras-e-Silva et al., 2017), it is currently unknown to what extent they elicit differential signaling profiles in the marmoset OTR. However, it is clear from this and previous studies that Leu⁸-OT and Pro⁸-OT differentially affect the expression of affiliation across multiple social contexts in marmosets.

While recent reviews have communicated important statistical and methodological concerns regarding the efficacy of intranasal neuropeptide treatments (Leng & Ludwig, 2015; Walum, Waldman, & Young, 2016), the effects of intranasal OT administration on social cognition and behavior are well-established (Quintana & Woolley, 2015). The recent surge of research aimed at determining the efficacy and mechanisms of intranasal OT in human and non-human primates (Chang et al., 2012; Dal Monte et al., 2014; Freeman et al., 2016; Lee et al., 2017; Modi, Connor-Stroud, Landgraf, Young, & Parr, 2014; Striepens et al., 2013) has

found that neuropeptides do not abundantly penetrate the BBB; it is estimated that 0.005% of the administered dose accumulates in the CSF within an hour (Leng & Ludwig, 2015). Thus, it may be that the fraction of intranasal OT that crosses the BBB accumulates in regions of the social behavior network to induce behavioral changes. Alternatively, elevated levels of peripheral OT following intranasal administration may result in central release of OT via afferent feedback pathways (Ferris et al., 2015; Quintana et al., 2015).

In the current study, the length of the separation was the main difference between Experiments 1 and 2, but they also differed on two other dimensions that may have contributed to the results. While adult heterosexual bonds are relatively stable and considered to be well-established following a two-month cohabitation in marmosets period (Ågmo, Smith, Birnie, & French, 2012; Schaffner et al., 1995), the difference in mean pair-cohabitation length between the long- and short-separation challenges may have contributed to the differential behavioral responses to separations upon reunion. Additionally, marmosets engaged in distinct tasks within each of these experiments that potentially contributed the behavioral responses upon reunion with their long-term pairmate; the long-separation challenged was designed to induce biobehavioral distress (Cavanaugh et al., 2016), while the short-separation challenge required marmosets to engage in a minimally stressful food-sharing task (Mustoe et al., 2015, 2016). Overall, our findings support the notion that not all social separations are equivalent, in degree or magnitude, and that marmosets have high levels of contextual sensitivity and the flexibility to adapt and respond to both subtle and major differences in social context during separation challenges.

Social separation, social isolation and social conflict are major sources of stress that when neglected, can contribute to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and adversely affect health and well-being (McEwen, 2008). High-quality partnerships and support systems are critical social resources, and serve as protective mechanisms against the deleterious outcomes associated with social stress (Carter, 1998; Cohen & Willis, 1985; Ditzen & Heinrichs, 2014; Wittig et al., 2016). Thus, reunion with a pairmate following a period of separation or isolation is important, not only to buffer against the detrimental effects of exposure to stressors, but also to re-establish social and physical proximity, cultivate attachment. The current results support the view that the OT system is important in social buffering and extend it by demonstrating that the OT system regulating *received* affiliative behavior following a long-separation challenge. Moreover, our results suggest that the OT system is sensitive to the context of separations and may transmit crucial social information from separations to reunion. Our results implicate OT as a bond-enhancing molecule that is fundamental to the reestablishment of optimal levels of affiliation with a mate following separation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

The design of the long-separation challenge (Experiment 1) and the short-separation challenge (Experiment 2) are displayed. In each experiment, marmosets received four drug treatments (oxytocin agonist Pro8-OT; oxytocin agonist Leu8-OT; oxytocin antagonist [OTA: L368,899]; saline control) across multiple social context conditions. Following intranasal drug administration, marmosets were transferred to a transport enclosure ($0.3 \times 0.3 \times 0.3$ m) and remained isolated in a quiet room some distance from the colony room that contained the pair's home enclosure for 30 min, after which behavioral testing commenced. Experiment 1 consisted of two social context conditions ([A] mate separation; [B] social isolation); exposure to each was 7 hr. Experiment 2 consisted of three social context conditions ([C] mate separation; [D] opposite-sex stranger separation; [E] social isolation); exposure to each was 15 min. Following each separation challenge, marmosets were reunited with their pairmate in their home enclosure.

FIGURE 2.

Mean ($\pm$ SEM) values for pair-directed proximity, subject-initiated proximity and grooming, and subject-received proximity and grooming behaviors following the long-separation challenge. Data in the left panels (a, c, e, g, i) are expressed as a function of drug treatment, collapsed across social-context: oxytocin agonist Pro8-OT; oxytocin agonist Leu8-OT; oxytocin antagonist (OTA: L368,899); saline control. Data in the right panels (b, d, f, h, j) are expressed as a function of social-context, collapsed across drug treatment (Grand mean):

social isolation; mate separation. Letters and asterisks indicate significant differences (a > b at $p < 0.05$, * at $p < 0.05$, # at $p < 0.10$)

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FIGURE 3.

Relationship between received gaze from social partners during short-separation challenge and time spent in proximity with a pairmate upon reunion. Data are expressed as a function of social context, collapsed across drug treatment (Grand mean): opposite-sex stranger separation ($\rho[7] = 0.73$, $p = 0.025$); mate separation ($\rho[7] = 0.23$, $p = 0.55$)

FIGURE 4.

Estimated mean ($\pm$ SEM) values for pair-directed proximity during reunion, adjusted for levels of social gaze during the short-separation challenge. Data are expressed as a function of drug treatment (oxytocin agonist Pro⁸-OT; oxytocin agonist Leu⁸-OT; oxytocin antagonist [OTA: L368,899]; saline control] and social context [mate separation; opposite-sex stranger separation). Letters indicate significant differences ($a > b$ at $p < 0.05$)

FIGURE 5.

Mean ($\pm$ SEM) values for received and initiated social gaze during the short-separation challenge. Data in the left panels (a and c) are expressed as a function of drug treatment and social-context: oxytocin agonist Pro⁸-OT; oxytocin agonist Leu⁸-OT; oxytocin antagonist (OTA: L368,899); saline control. Data in the right panels (b and d) are expressed as a function of social-context, collapsed across drug treatment (Grand mean): social isolation; mate separation; opposite-sex stranger separation. Letters indicates significant differences (a > b at $p < 0.05$, # $p < 0.10$). Asterisks indicate significant differences at $p < 0.05$

TABLE 1

Ethogram

Behavior	Operational definition
Proximity ^{a,b}	Duration that marmoset spends within 10 cm of mate
Approach ^a	Moving to a distance of <10 cm from mate
Leave ^a	Moving to a distance of >10 cm from mate
Huddle ^a	Duration that pair spends sitting in physical contact
Solicit groom ^a	Orientation of body or head to present to mate for grooming
Allogroom ^{a,b}	Manipulation of pelage of mate by parting the fur with hands and removing particles with hands or teeth
Autogroom ^a	Manipulation of pelage of self by parting the fur with hands and removing particles with hands or teeth
Scent mark ^a	Anogenital rubbing across a substrate, often preceded by gnawing on surface, or co-occurring with urination
Mount ^a	Male grasps female's back and thrusts pelvis with an erect phallus
Social gaze ^c	Duration of head orientation where eyes are directed toward other individual
Social proximity ^c	Duration of time spent on the nearest half of the enclosure adjacent to the other individual

Behaviors observed during:

^aReunion following the long-separation challenge,

^bReunion following the short-separation challenge,

^cShort-separation challenge.