

Vasopressin, but not oxytocin, modulates responses to infant stimuli in marmosets providing care to dependent infants

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Abstract

In family-living species, the quality and patterning of caregiving is the product of an individual's role within the family (mother, father, sibling) and parental experience, both of which interact with underlying neurobiological substrates. Among these substrates are the nonapeptides vasopressin and oxytocin, which modulate maternal, paternal, and alloparental care. We used a nonhuman primate model of the "nuclear family," the marmoset (*Callithrix jacchus*), to investigate relationships between caregiving experience, role within the family, and activation of either the oxytocin or vasopressin systems in shaping responsiveness to offspring. During two phases of offspring development (early infancy, juvenile), mothers, fathers, and older siblings were treated with vasopressin, oxytocin, or saline via intranasal application, and tested for responses to infant distress stimuli in a within-subjects design. Interest in infant stimuli was highest among marmosets that were caring for infants compared to those caring for juveniles, and parentally experienced marmosets were quicker to respond to infant stimuli than first-time caregivers. Moreover, marmosets treated with vasopressin showed enhanced responsiveness to infant stimuli compared to control stimuli only when caring for infants. Thus, in all classes of marmoset caregivers, vasopressin enhances responsiveness to infant-associated stimuli in caregivers during periods in which infant care is most crucial.

KEYWORDS

alloparental care, development, intranasal, oxytocin, parental care, primate, social behavior, vasopressin

1 | INTRODUCTION

The birth of a child and the period of intensive infant care after parturition represent a significant change in the patterns of social interactions within the family for first-time parents, experienced parents, and older siblings alike (Nyström & Öhrling, 2004; Volling, 2012). Among many nonhuman mammals, parturition itself is a critical precipitating event that initiates mother-offspring bonding and maternal behavior in individuals that had never expressed such behavior before (Kendrick, Lévy, & Keverne, 1991; Keverne & Kendrick, 1992; Stolzenberg & Champagne, 2016). However, the physical act of giving birth is not the only event that can change behavior from

nonparental to parental. In paired California mice, first-time experience as a father results in enhanced caregiving behavior compared to non-fathers, and experience with a second litter enhances caregiving behavior even further (Gubernick, Winslow, Jensen, Jeanotte, & Bowen, 1995; Perea-Rodriguez et al., 2015). Fatherhood is associated with multiple measures of enhanced responsiveness to infant stimuli in marmoset monkeys as well. When compared to inexperienced non-fathers, parentally experienced marmoset fathers are faster to enter a novel cage containing infant stimuli and engage in more stimulus-directed behavior than non-fathers (Zahed, Prudom, Snowden, & Ziegler, 2008; Ziegler & Sosa, 2016). Moreover, simple exposure to unrelated young influences the development of

caregiving behavior in non-parents. Sexually naïve female rats and mice initially fail to care for unrelated pups, but repeated exposure to pups will show spontaneous caregiving behavior (Okabe, Kitano, Nagasawa, Mogi, & Kikusui, 2013; Okabe et al., 2017; Rosenblatt, Hazelwood, & Poole, 1996; Stolzenberg & Champagne, 2016). Similarly, parentally inexperienced male marmosets initially show an inhibited response to infants and infant stimuli, but with repeated exposure rapidly increase their approach and caregiving behavior to equally experienced marmosets (Barbosa, Mota, & Barbosa, 2017; Barbosa & Da Silva Mota, 2013). Thus, exposure to and experience caring for young are critical determinants of caregiving behavior, even in those individuals that do not experience pregnancy and parturition themselves.

Among cooperatively breeding species, there are many opportunities to care for young (Barbosa & Da Silva Mota, 2013; Kenkel, Perkeybile, & Carter, 2017; Yamamoto, 1993), and this experience with caring for siblings impacts responses to infants and infant stimuli. In marmosets, the determining factor in altered behavior toward an infant is not whether a male has parented his own offspring before, but whether he has experience in caring for siblings, with males with alloparental experience exhibiting higher levels of spontaneous caregiving and those that did not (Barbosa & Da Silva Mota, 2013). Experience with caregiving often confers benefits to the older sibling alloparent in terms of increased future offspring outcomes in cooperatively breeding rodents as well as cotton-top tamarins and marmosets (Salo & French, 1989; Stone, Mathieu, Griffin, & Bales, 2010; Tardif, Richter, & Carson, 1984). These data show that experience with siblings, often before sexual maturity, enhances later responses to young after maturation, mating, and parenthood.

Exposure to young clearly produces changes in caregiving behavior, but there remains a question of whether these changes are permanent and stable, or are dynamic and dependent on the individual's current demand for caregiving behavior. Experience effects, in particular those that demonstrate enhancements in care from the first litter to the second (Barbosa et al., 2017; Barbosa & Da Silva Mota, 2013; Perea-Rodriguez et al., 2015) or those that result from exposure to younger siblings (Kelley, Castelli, Mabry, & Solomon, 2013; Salo & French, 1989; Stone et al., 2010; Tardif et al., 1984) suggest that these changes are permanent. However there is evidence that caregiving behavior, and the underlying neurobiology that mediates these changes, are responsive to differences in parenting demand. The nonapeptides oxytocin (OT) and arginine vasopressin (AVP) are two hormones that appear to track with changing caregiving demand. Plasma OT is elevated during the first 6 months of caregiving in human mothers and fathers (Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010a) *c.f.* (Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010b), and is positively associated with affectionate touch and affective synchrony in parent-infant dyadic interactions (Apter-Levi, Zagoory-Sharon, & Feldman, 2014; Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Gordon, & Zagoory-Sharon, 2011; Gordon et al., 2010b). A similar increase in peripheral OT is present in marmosets, and OT is positively associated with infant-care behaviors (Finkenwirth,

Martins, Deschner, & Burkart, 2016). Likewise, AVP V1a receptors are upregulated in the forebrain of marmoset fathers after the birth of offspring, and receptors are downregulated as infants mature and require less care (Kozorovitskiy, Hughes, Lee, & Gould, 2006). These data suggest that the OT and AVP systems modulate caregiving behavior according to caregiving demand. However, OT and AVP also appear to be involved in more permanent changes in caregiving behavior. An OT receptor antagonist blocks the facilitating effect of pup exposure on caregiving in female mice, but only if infused during initial exposure to pups. Female mice previously exposed to pups provided care regardless of whether OT receptors were blocked (Okabe et al., 2017). Experience likely causes long-lasting changes in the AVP system as well. Variation in the microsatellite region of the AVP V1a receptor gene is associated with caregiving behavior in prairie voles (Hammock & Young, 2002), but this gene-behavior association is present only during the first litter, and is not present in experienced caregivers (Kelley et al., 2013). Since providing quality caregiving is important for offspring development, and because there is variability in the effects of experience on caregiving behavior (Kelley et al., 2013), it is critical to understand the neurobiological mechanisms that mediate the relationships between caregiving experience and the expression of quality care, particularly in species with complex social behavior and long developmental histories.

The marmoset monkey is a useful model to explore the relationships between role within the family (mother, father, sibling), parental experience, and the neuroendocrine mediation of parental care by OT and AVP. Marmoset families are optimally housed as a breeding pair with dependent and independent offspring. Both males and females exhibit neuroendocrine and behavioral changes across pregnancy and caregiving (Saltzman & Ziegler, 2014; Ziegler, Prudom, & Zahed, 2009). The OT and AVP systems dynamically track the caregiving needs of offspring. AVP V1a receptors are upregulated in the forebrain of marmoset fathers after the birth of offspring, and receptor density in fathers declines as infants mature and require lower levels of care (Kozorovitskiy et al., 2006). Similarly, estrogen-inducible OT release from the hypothalamus is higher in fathers than in non-fathers, but this change is stable across infant ages, rather than waning as infants become independent (Woller et al., 2012). Lastly, in marmoset caregivers of all types (mothers, fathers, siblings), the specific caregiving behaviors that are positively associated with urinary OT change across time to reflect the needs of the care-recipient (Finkenwirth et al., 2016). These studies show that the nonapeptide systems in marmosets are highly sensitive to changing caregiving needs.

In this paper, we examined whether exogenous OT and AVP had differential effects on responsiveness to infant stimuli in marmosets, based on the current effort in parental care (presence vs. absence of dependent infants in the family group). We tested this proposition in adult parents and subadult alloparents, and whether they were experienced in parental care or inexperienced. Caregivers (alloparents or parents) were treated with OT, AVP, or saline control, and then tested with an infant model emitting distress calls and a control non-social stimulus, either when highly dependent infants were present

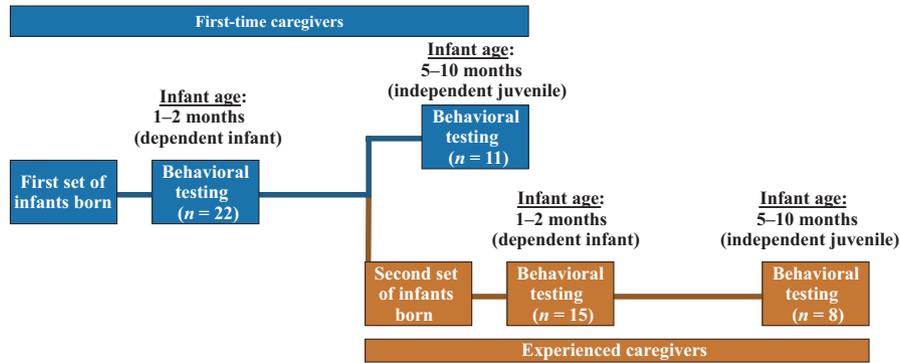


FIGURE 1 Experimental timeline. Behavioral testing after treatment with vasopressin, oxytocin and saline began 1–2 months after the birth of offspring. Each caregiver (mothers, fathers, older siblings) received all three treatments. For a subset of the sample, treatment and behavioral testing was repeated with the subsequent successful litter. A final treatment and testing phase began when the youngest family members were 5–10 months of age

in the family (high demand for infant care), or after those same infants had grown to be largely independent juveniles (low demand for infant care). If the OT and AVP systems are stably altered after experiencing caregiving for the first time, then marmosets should be more sensitive to nonapeptide manipulation after caring for at least one litter. If, in contrast, OT and AVP systems are transiently altered by the act of engaging in high levels of offspring care, we predicted that marmosets would be more sensitive to nonapeptide manipulations only when dependent infants are present. Lastly, if OT and AVP affect responsiveness to infant distress stimuli nonselectively through a change in neophobia or interest in novel stimuli, then nonapeptide manipulation should enhance or inhibit investigation of infant and control stimuli equally.

2 | METHODS

2.1 | Subjects and observational timeline

We used eight families of marmosets (*Callithrix jacchus*) in this study (see Table S1 for sample demographics). All families were composed of a male and a female breeder and their offspring. There were 12 successful pregnancies (resulting in at least one surviving infant) during the course of the study. Offsprings were kept in the natal group until at least 18 months of age, and families were kept intact as long as possible. Observations were conducted at two time points that reflect differential direct involvement in offspring care (Figure 1). The first set of observations occurred when youngest offspring in the family group were approximately 1 month of age ($M = 35.8$ d, $Range = 17$ – 60 d), a time point at which both parents and siblings are actively involved in offspring care in marmosets (Nunes, Fite, Patera, & French, 2001; Tardif, Carson, & Gangaware, 1986; Ziegler et al., 2009). The second set of observations occurred when infants were no longer nursing on mothers or being actively carried or provisioned by caregivers (5–10 months of age; $M = 228.2$ d, $Range = 146$ – 285 d). Marmosets were considered first-time caregivers if they were providing care for their first litter (breeders) or their first set of infant siblings (alloparents), and were considered experienced for all

subsequent litters/siblings. All animal procedures were approved by the University of Nebraska at Omaha IACUC (#17-071-09-FC).

2.2 | Nonapeptide treatments and experimental procedure

On the day of testing, marmosets were caught and treated via intranasal application with the variant of OT native to marmosets (Lee et al., 2011; Ren et al., 2015; Vargas-Pinilla et al., 2015), Proline-8-OT (Pro⁸-OT; CYIQNCPPG-NH₂; approximately 150 µg/kg), AVP (CYFQNCPRG-NH₂; approximately 133 µg/kg), or saline. Marmosets were captured from the home cage, gently manually restrained, and 75–100 µl of peptide solution was pipetted dropwise into both nostrils. All marmosets were tested on three different days per time point, one test for each treatment. Treatments and tests for each marmoset were counterbalanced, spaced 24–96 hr apart, and only one member of each family was tested each day. Mothers and fathers completed all treatments first over the course of 4–10 days, followed by sibling pairs in descending order of age. This order of testing was chosen in accordance with the timing of infant carrying effort among different classes of caregivers (French, Fite, & Ross, 2008).

After receiving one of the nonapeptide treatments, each marmoset was transferred to a transport cage (30 × 30 × 30 cm) for 20 minutes for uptake. In macaque monkeys, plasma OT is elevated 10 min after intranasal treatment, and CSF levels are elevated 30–40 min post-treatment (Chang, Barter, Ebitz, Watson, & Platt, 2012; Monte, Noble, Turchi, Cummins, & Averbeck, 2014). Assuming similar pharmacokinetics in marmosets, behavioral testing occurred at a timepoint when central and peripheral neuropeptide levels are predicted to be elevated. At the end of uptake, the marmoset was released into a large T-maze. Four 42 cm zones were marked along the long arm of the T, and one 42 cm zone was marked at the start of the short arm. At each end of the long arm of the T-maze were opaque stimulus boxes with two small viewing holes (2 × 2 cm). One stimulus box contained a lifelike model of two infant marmosets and a speaker that played infant distress

(nga) and contact (phee) calls (15 total calls). Infant distress calls were recorded from four infants aged 2 weeks old that were unrelated to any of the subjects. Infants were gently held by an experimenter and were recorded with a microphone capable of recording at frequencies between 0 and 21,000 Hz. The other stimulus box contained a branch and a speaker that played 15 s control tones at 6,000 Hz, the approximate length and dominant frequency of the infant nga call (Taylor & French, 2015). The duration of each auditory stimulus was 30 s, with a 5 s period of silence between stimulus types (infant or control). This apparatus allows marmosets to localize auditory stimuli and provides no visual access unless the subject is close to the viewing holes, and only limited visual access when using the viewing holes to see within the darkened box. Once released into the T-maze, marmosets were able to freely move about the apparatus for 10 min. The critical behavior for interest in stimuli was "peeking", which was defined as looking into the stimulus boxes with the face less than ~10 cm from the viewing hole. We recorded the latency to the first peek in each box, the frequency of peeks, and the total duration of peeking. We also recorded anxiety-related behaviors: sector crossing (distinct movements from one of the five 42 cm zones of the T-maze to another), vocalizations (contact/phee calling and alarm calling), and neophobia (latency to peek into the novel control box). Phee calling and alarm calling were only scored when audio quality allowed (~91% of all trials).

2.3 | Statistical analysis

A nested linear mixed effects model was used to evaluate the main and interactive effects of nonapeptide treatment, sex, social role (parent vs. sibling), parental experience (first-time caregiver vs. experienced), and presence of dependent infants (dependent infants vs. independent juveniles) on marmoset behavior (Equation 1). Nonapeptide treatment conditions were nested within individuals, and individuals were nested within families. Only significant main effects and interactions including stimulus type were probed, except when vocalization or sector crossing were the dependent variables. Moreover, only the highest order interaction containing nonapeptide treatment as well as the highest order interactions not containing treatment were probed. Significant main effects and interactions were explored using Fisher's *post hoc* tests, using a Satterthwaite approximation for degrees of freedom. Omnibus tests and *post hoc* tests were considered significant if $p < 0.05$. All data are presented as model estimated marginal means (M) and model standard errors of measurement (SEM).

$$\text{Behavior} = \text{Caregiver Sex} \times \text{Treatment} \times \text{Social role} \times \text{Caregiver experience} \times \text{Infant presence} \times \text{Stimulus Type}^{\#} + \text{Error}(\text{Treatment}) + \text{Error}(\text{Animal ID}) + \text{Error}(\text{Family ID})$$

Equation 1, Template model for analysis of behavioral data. # indicates this factor was not included when vocalizations or sector crossing were the dependent variable, as they were not directed toward the stimuli.

3 | RESULTS

3.1 | Responses to infant and control stimuli

In general marmosets were more interested in infant stimuli than control stimuli. There was a main effect of stimulus type for peek count ($F_{1,315.7} = 11.40, p < 0.001$), peek duration ($F_{1,315.4} = 10.65, p = 0.001$), and peek latency ($F_{1,314.12} = 6.31, p = 0.013$). Marmosets were faster to investigate infant stimuli (Infant stim: $M(\pm SEM) = 225 (31.7)$ s; versus Control stim: 276 (31.7) s), investigated infant stimuli for longer total durations (Infant stim: 42 (0.14) s; versus Control stim: 0.25 (0.14) s), and more often (Infant stim: 0.31 (0.06); versus Control stim: 0.23 (0.06) s) than control stimuli.

3.2 | Responses to stimuli across social classes and family contexts

The time and frequency that marmosets spent investigating infant-related versus novel stimuli differed based on whether caregivers were providing care to infants or to juveniles in their own families (duration: $F_{1,315.4} = 7.95, p = 0.005$, Figure 2a; frequency: $F_{1,315.7} = 4.08, p = 0.044$, Figure 2b). In general, interest in infant stimuli was greater when dependent infants were present in the family compared to after infants had grown into juveniles. Marmosets caring for infants spent more time peeking into the infant stimulus box compared to the time spent peeking into the control stimulus box, or compared to time spent peeking in the infant stimulus box after the youngest family members had grown to juveniles. Marmosets caring for infants also peeked in the infant stimulus box more often than they peeked in the control box, whereas marmosets caring for juveniles did not peek in either box significantly more often. Moreover, the latency to peek in the two stimulus boxes depended on the caregiver's experience ($F_{1,314.12} = 5.53, p = 0.019$; Figure 3). Experienced caregivers, regardless of whether they were providing care to infants or juveniles, were faster to peek into the infant stimulus box compared to the control stimulus box, whereas first-time caregivers did not peek in one box any faster than the other box. Status as a breeder versus alloparent was not associated with differences in stimulus-directed behavior (all $F_s < 2.50$, all $p_s > 0.10$).

3.3 | Effects of vasopressin and oxytocin

Nonapeptide treatments modified responses to test stimuli depending on the age of the youngest members of the family ($F_{2,314.12} = 3.26, p = 0.040$; Figure 4). When they were caring for infants, marmosets treated with AVP peeked in the infant stimulus box faster than they peeked in the control stimulus box (Figure 4a). In contrast, after their infants had grown to juveniles, caregiver marmosets treated with OT were marginally slower to peek in the infant stimulus box compared to the control box (Figure 4b). Moreover, when juveniles were present in the family, marmosets

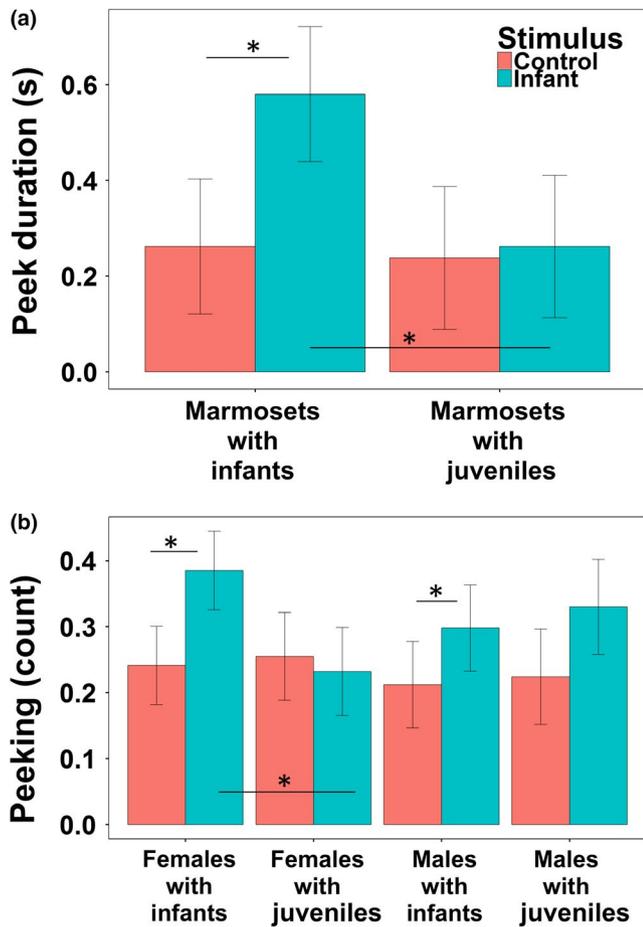
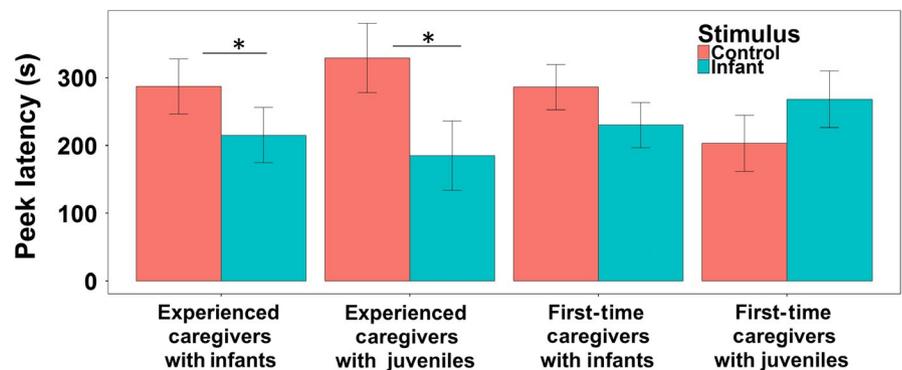


FIGURE 2 Model estimated marginal mean (\pm SEM) (a) duration and (b) frequency of “peeking” into the stimulus boxes. Bars connected by lines and asterisks are significantly different. *Post-hoc* differences marked with * indicate $p < 0.05$. Means (\pm SEM) in a) are collapsed across treatment, sex, social role, and experience. Means (\pm SEM) in b) are collapsed across treatment, social role, and experience

treated with saline were faster to peek in the infant stimulus box compared to the control box. However, latency, duration, or frequency of peeking into the infant stimulus box did not differ between marmosets treated with OT or AVP compared to when treated with saline.

FIGURE 3 Model estimated marginal mean (\pm SEM) duration “peeking” into the stimulus boxes. Bars connected by lines and asterisks are significantly different. *Post-hoc* differences marked with * indicate $p < 0.05$. Means (\pm SEM) are collapsed across treatment, sex, and social role



3.4 | Anxiety-related behaviors

OT and AVP did not affect the expression of alarm calls, phee calls, or all vocalizations combined emitted by marmosets during the trials (all F 's < 3.02 , all p 's > 0.05). However, treatment with nonapeptides did interact with experience with caregiving and the age of the youngest family members to affect locomotion in the form of sector crossing ($F_{2,145.0} = 4.19$, $p = 0.017$; Figure 5). When providing care to juveniles, first-time caregivers treated with AVP engaged in more sector crossing than when they were treated with saline. As a measure of generalized neophobia, peeking in the control stimulus box was compared among treatments for the interactions depicted in Figure 4. Compared to saline treatment, OT reduced the latency to peek in the control stimulus box in marmosets providing care to juveniles, but no significant difference was present in those caring for infants.

4 | DISCUSSION

The purpose of this experiment was to test whether exogenous OT and AVP altered responsiveness to infant stimuli in marmosets as a function of level of current engagement in infant care. Further, we evaluated whether the neuropeptide-behavior relationships varied as a function of role in the family group (parents vs. alloparents) and levels of previous experience (experienced and inexperienced). Enhanced responsiveness to nonapeptides can occur in several ways, such as an increase in receptor number, a stable increase in nonapeptide concentration, and/or an increase in nonapeptide release following exposure to a social stimulus. We hypothesized that if the OT and AVP systems are permanently altered after experiencing caregiving for the first time, then marmosets would be more sensitive to nonapeptide manipulation after experience with infants. This hypothesis was not supported. First-time marmoset caregivers did not differ in their responses to infant stimuli after OT or AVP treatment from those that had cared for two or more litters. An alternative hypothesis posited that if the OT and AVP systems are sensitized only when actively caring for dependent infants, marmosets will be more sensitive to nonapeptide manipulations only when caregivers were actively engaged in parental care with young infants. This hypothesis was

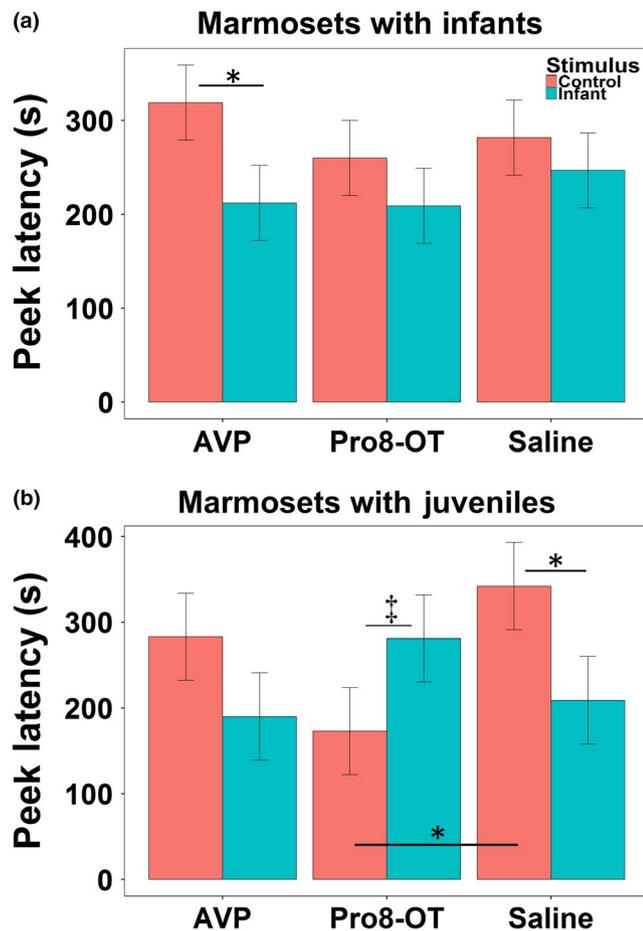


FIGURE 4 Model estimated marginal means (\pm SEM) latency "peeking" into the stimulus boxes in marmosets a) with infants present in the family and b) those with juveniles present in the family. *Post-hoc* differences marked with * indicate $p < 0.05$. *Post-hoc* differences marked with ‡ indicate $p < 0.10$. Means (\pm SEM) are collapsed across sex, social role, and experience

partially supported. In marmosets caring for infants, AVP treatment decreased the latency to investigate infant stimuli relative to control stimuli. However, in marmosets caring for juveniles, OT marginally increased the latency to investigate infant stimuli relative to control stimuli.

An explanation for the fact that neither of the hypotheses were fully supported may be that AVP and OT altered responses to a potentially anxiety-inducing testing situation in addition to infant-specific responses. Exposure to infant vocalizations alone increases plasma cortisol in marmosets (Barbosa et al., 2017), and it is likely that the capture and removal from the home cage induced some level of stress on the subjects. In general, OT tends to act as an anxiolytic and AVP tends to be anxiogenic and aggressogenic (Neumann, 2008; Neumann & Slattery, 2016; Takayanagi et al., 2005; Viviani & Stoop, 2008), though there are important sex and species differences in the expression of aggression (Been, Gibbons, & Meisel, 2018; Terranova, Ferris, & Albers, 2017). Aggression is an important component of parental behavior, as protection of offspring from conspecifics often requires aggressive responses by parents, and this parental aggression is influenced by anxiety and the OT and AVP systems in rodents (Bosch & Neumann, 2012; Nephew & Bridges, 2008; Nephew, Byrnes, & Bridges, 2010; Trainor, Finy, & Nelson, 2008). In light of these rodent data, it is useful to interpret studies involving OT and AVP in marmosets within the framework of aggression and anxiety, as well as within the framework of prosociality. Marmoset fathers refuse to share food to weaned and food-independent juveniles more often when treated with AVP compared to saline, and among all marmoset caregivers, AVP increased aggressive vocalizations toward juveniles in a food-sharing task (Taylor, Intorre, & French, 2017). This suggests that the increase in food refusals in AVP-treated marmoset fathers may be mediated via food-related aggression. It is also likely that the experience of caring for offspring affects the sensitivity of marmosets to the anxiogenic and anxiolytic properties of OT and AVP. In a sample composed mainly of non-parent marmosets, OT and AVP enhanced responsiveness to infant stimuli, but not control stimuli (Taylor & French, 2015). However, the current study demonstrated that OT and AVP affected responses to both classes of stimuli, in opposing directions. In marmosets not actively caring for infants, but with juveniles in the family, OT reduced neophobia directed toward a novel control stimulus, while simultaneously marginally inhibiting responsiveness to infant stimuli. Likewise, in marmosets actively caring for infants, AVP treatment altered responses to both infant and control stimuli, such that AVP-treated marmosets investigated infant stimuli faster than they investigated control

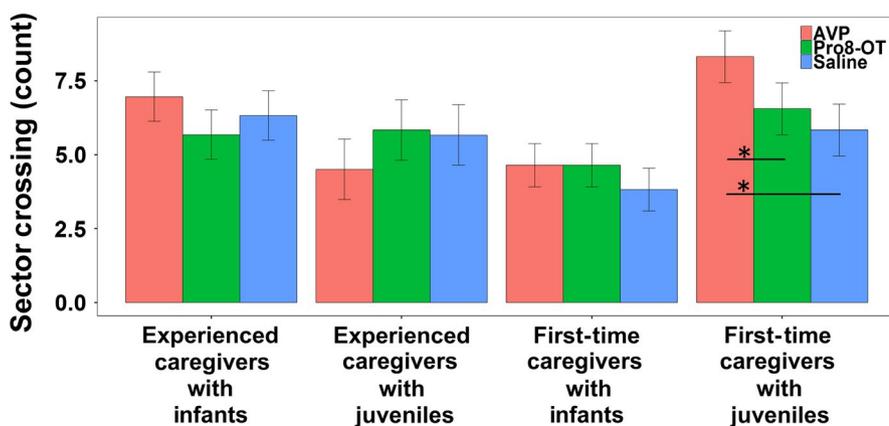


FIGURE 5 Model estimated marginal mean (\pm SEM) sector crossing. *Post-hoc* differences marked with * indicate $p < 0.05$. Means (\pm SEM) are collapsed across sex and social role

stimuli, whereas saline-treated marmosets did not differ in their responsiveness to infant and control stimuli. Moreover, AVP increased general locomotion in first-time marmoset caregivers that were not actively caring for infants. These results suggest that the systems that control general anxiety/neophobia and those that control responses to infants are each sensitive to nonapeptide manipulation, and both undergo transient change according to the developmental stage of their youngest family members.

The results from this study suggest that the OT and AVP systems undergo transient change depending on whether or not marmosets are delivering care to dependent infants or to independent juveniles. This pattern of temporary neuroendocrine reorganization in response to parental demands is well-documented across species. For instance, marmosets that are actively caring for young have an increased labeling of V1a receptors in the prefrontal cortex while they are caring for offspring, and the degree of V1a receptor labeling is negatively associated with the age of offspring (Kozorovitskiy et al., 2006), suggesting that upregulation of V1a receptors is temporary and in response to active infant caregiving. In ewes, plasma AVP and CSF OT concentrations are increased during contractions and birth and remain elevated temporarily post-partum during a period of time that is known to be critical to ewe-lamb bonding (Kendrick, Keverne, Hinton, & Goode, 1991). Further evidence of the importance of transient and dynamic changes in neuropeptide systems for parental behavior comes from studies of parent–infant interactions in humans. Mothers that demonstrate high levels of affection with their infants have increased levels of OT after interaction with their babies, while mothers that display low levels of affectionate behavior toward their infants do not exhibit this increase in OT post-infant interaction (Feldman et al., 2010). Likewise, fathers show increased OT concentrations after engaging in high levels of stimulatory contact with their infants (Feldman et al., 2010). Common marmosets show a similar pattern of relationship between quality of interaction with infants and neuropeptide system responses. In both parents and alloparents, urinary OT levels are increased after infant birth and are positively correlated with degree of caretaking behavior exhibited by the individual (Finkenwirth et al., 2016). Together, these findings highlight the way in which active interaction with an infant can potentially increase activity of the OT system in parents. Importantly, during periods of high paternal care, administration of OT reduces the number of times that marmoset fathers refuse to share food with their offspring, indicating that OT increases tolerance of males currently caring for dependent offspring, but not when offspring are older (Saito & Nakamura, 2011). Furthermore, both OT and AVP reduce food sharing behavior to older and independent siblings and offspring in male marmosets (Taylor et al., 2017), indicating the effects of OT and AVP on caregiving behaviors depend on recipient age. In the current study, we found clear differences in responses to infant stimuli when caregivers were actively providing care to infants compared to after those infants had grown to juveniles. The effects of nonapeptide treatment on responsiveness to infant stimuli were coupled with treatment-induced changes in anxiety-related behavior, and were also dependent on

the presence or absence of infants in the family. Thus, our data and those of others show that marmosets undergo transient changes in the OT and AVP systems during periods of infant care and development. These flexible changes to the system highlight plasticity in key neural systems involved in the changing demands of parental care.

In our previous study, the sample of marmosets used was mostly composed of non-parents (Taylor & French, 2015), while the marmosets studied in the current study were first-time and experienced parents and alloparents. As such, the behavior observed in these two experiments can potentially be viewed as a developmental trajectory from spontaneous interest in infant stimuli in nulliparous marmosets, to the experience and first-time caregiving, to experienced caregiving. In our previous study, nonapeptide-induced changes in responses to infant and control stimuli were entirely attributable to alterations in responsiveness to infant stimuli, and not generalized anxiety-like behavior or neophobia. However, in the current study, in addition to its effects on responsiveness to infant stimuli, AVP increased locomotor activity and OT decreased neophobia to a nonsocial stimulus. Moreover, there was a clear sex difference in our previous studies in the effect of nonapeptide treatment; OT and AVP enhanced responsiveness to infant stimuli in males and females, respectively (Taylor & French, 2015), and OT and AVP each affected food sharing behavior in male, but not female, caregiver marmosets (Taylor et al., 2017). However, no sex differences were found in the current study. Instead, AVP enhanced responsiveness to infant stimuli relative to control stimuli in all caregivers, but only when caregivers had infants present in the family. Similarly, OT treatment inhibited responsiveness to infant stimuli in families with only juveniles in the family, regardless of sex. Rather than being dependent on sex or social role, differences in nonapeptide-induced behavior were context dependent. This context-dependent hypothesis for the effects of OT and AVP on caregiving behavior is supported by the finding that urinary OT in marmosets is associated with different infant-care behavior depending on the age and needs of the infant (Finkenwirth et al., 2016), as well as the finding that both the neural AVP system and the behavioral response to OT treatment in marmoset fathers changes with the age of infants (Kozorovitskiy et al., 2006; Saito & Nakamura, 2011). These context-specific results highlight the value of studying caregivers as units within a larger context, such as a family, and the value of accounting for important social and developmental variables such as age, sex, and role within the family. These social factors interact with the nonapeptide systems to produce context-appropriate responses to infant stimuli in family-living marmoset caregivers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (JHT) upon reasonable request.

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