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The role of father-daughter relationships in mediating behavioral, physiological, and neural mechanisms underlying attachment in female titi monkeys (*Plecturocebus cupreus*)

By

LYNEA RENEE WITCZAK
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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OFFICE OF GRADUATE STUDIES

of the

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DAVIS

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GENERAL ABSTRACT

Social interactions regulate our behavior and physiology (Uchino, 2006), and connections between social well-being and health may be one reason why individuals who are happy with their relationships live longer (Diener & Chan, 2011). A pair bond is a selective, enduring, relationship that is regulated by physiology and behavior (Bales et al., 2021). Understanding mechanisms underlying social bonds is especially critical now because the COVID-19 pandemic has detrimentally impacted human social relationships (Evans et al., 2020; Luetke, Hensel, Herbenick, & Rosenberg, 2020), which may result in adverse health outcomes (Saggioro et al., 2021). Despite the importance of social bonds for well-being, little is known about their underlying neurobiology. Much of what we know about the neurobiology of attachment comes from research conducted on males (Bales et al., 2017), despite studies finding opposite behavioral responses to neuroendocrine manipulations in males and females (Carter, Boone, & Bales, 2008). Women are more likely to develop affective disorders like depression, which may be linked to social risk factors. Research on female attachment, particularly studies on daughters' relationships with fathers, is incredibly limited and therefore warrants further investigation.

Nonhuman primates are excellent models for understanding the effects of social bonds on human health due to their similar social dynamics, cognitive functioning, and immune systems (Phillips et al., 2014). Coppery titi monkeys (*Plecturocebus cupreus*) are socially monogamous South American monkeys that live in small family groups. They display classic attachment behaviors (Fuentes, 1998), including proximity maintenance with (Carp et al., 2016), distress upon separation from (Mendoza & Mason, 1986b), and stress buffering by (Mendoza, Capitanio, & Mason, 2000) the attachment figure. Partners are each other's primary attachment figures,

while infants form selective bonds with their fathers (Mendoza & Mason, 1986b, 1986a), making them ideal for studying father-daughter bonds.

Methods: Our main objectives were to 1) identify foundational neurobiological and behavioral mechanisms underlying female attachment relationships and 2) determine how relationship quality affects bonding mechanisms. We established a novel method for measuring relationship quality in titi monkeys by quantifying degree of proximity maintenance and separation distress exhibited during infancy and adolescence in females. Given the importance of oxytocin (OT) and arginine-vasopressin (AVP) in pair bonds (Lim & Young, 2006), we also investigated how manipulations of these neuropeptides influenced juvenile attachment. Subjects received acute intranasal treatments of saline, low/medium/high OT, low/high AVP, or OT antagonist (OTA) prior to a social separation or parent preference test (Carp et al., 2016). These same females were then paired as adults and underwent preference tests and Positron Emission Tomography (PET) scans to determine how father-daughter bonds impact adult attachment. We used stepwise regression to determine which measures of infant and juvenile attachment best explained variability in responses to our social challenges. We then investigated the effects of predictors of relationship quality and neuropeptide treatments using general linear mixed-effects models and zero-inflated mixed-effects models with Poisson distributions.

Chapter 1 Results: Fathers were significant behavioral and physiological stress buffers for their daughters as evidenced by fewer distress vocalizations ($p < .001$), less locomotion ($p < .001$), and lower plasma cortisol ($p < .001$) during social separation testing. Daughter's quality of relationship with their fathers significantly explained variability in subjects' separation distress. For example, females produced fewer distress vocalizations if they spent more time in proximity to their fathers as infants ($p = .03$), and this stress buffering effect remains even when the

daughter is separated from the father ($p < .001$). While treatments did not alter separation distress behaviors, blockade of OT receptors with OTA resulted in the largest rise in plasma cortisol ($p < .001$), suggesting OTA is able to block the stress-buffering effects fathers have on daughters. Remarkably, if females have a high-quality father-daughter relationship, they exhibit an overall reduced physiological response to social separation ($p = .04$).

Chapter 2 Results: Across all parent preference tests, females spent more time in proximity to their parents than the strangers or in the neutral preference zone; however, females exhibited a large degree of individual variation. Manipulations of the OT and AVP systems drove increased proximity maintenance with parents for females that exhibit behaviors indicative of a higher-quality relationship with their fathers. For example, females that spent more time in proximity to their fathers as infants also spent more time in proximity to their parents when treated with Medium OT ($p = .007$) and High AVP ($p = .002$). Females with higher quality father-daughter relationships also spent less time in proximity to strangers when treated with High OT ($p = .003$) and Low OT ($p = .007$), but more time near the strangers when treated with High AVP ($p = .007$), Low AVP ($p = .009$), and OTA ($p = .001$). These findings suggest that when females exhibit behaviors indicative of a high-quality relationship with their fathers, activation of the OT system reduced willingness to interact with strangers, while blockade of OT receptors and activation of the AVP system dysregulated species-typical proximity behaviors.

While OT and AVP treatments did not impact the separation distress behaviors measured, the father-daughter relationship quality did. Females locomoted more during testing if they spent more time in proximity to their fathers as infants ($p < .001$) and juveniles ($p = .03$) and exhibited more distress upon separation as juveniles ($p < .001$). These findings suggest other neuroendocrine systems may be driving separation distress behaviors.

Chapter 3 Results: Across our two experiments for Chapter 3, we found evidence to suggest that measures of relationship quality significantly explain variability in behavioral and neural correlates of adult social bonds in female titi monkeys. In particular, a higher-quality relationships with the father established a stronger foundation for forming and maintaining an adult pair bond. We also found evidence to suggest that females are capable of maintaining a relationship with their father at least 6-months post pairing, as evidenced by females spending a greater percentage of time in proximity to their fathers during testing if they exhibit higher-quality relationships with their fathers. Our examination of neural activity pre- and post-pairing suggests a high degree of overlap between circuitry driving filial attachment and adult pair bonding, as patterns of activity were similar across the two time points, despite overall activity being lower post-pairing ($p < .001$). It is likely that differences in OT, AVP, dopamine, and opioid activity underlie the differences observed in neural activity based on daughter's relationship with their fathers.

Conclusions: Overall, variability in the quality of father-daughter relationships significantly predicted patterns of stress buffering, separation distress, and proximity maintenance, while manipulations of the OT and AVP systems specifically impacted physiological separation distress and proximity maintenance. While blockade of OT receptors increased plasma cortisol, females with higher-quality father-daughter relationships exhibited reduced physiological and behavioral separation distress responses. Because our manipulations of the OT and AVP systems did not significantly affect separation distress behaviors, it is possible that other neuroendocrine systems, such as dopamine and opioids, influence female separation distress. Activation of the OT system increased time females spent in proximity to their parents during preference testing, but only if females also exhibited behavioral indicators of

high-quality father-daughter relationships. Interestingly, activation of the AVP system and blockade of OT receptors resulted in dysregulated species-typical behavior, where females were more willing to interact with strangers. Females' responses to treatments were mediated by their relationships with their fathers, suggesting differences in relationship quality may alter OT and AVP receptor distributions. For example, females with a higher-quality father-daughter relationship may have a higher density of OT receptors, which may make them more sensitive to activation of the OT system. Females with a higher-quality relationship with their father also exhibited a stronger foundational framework for forming and maintaining adult pair bonds. We also found evidence to suggest that daughters' preference for their fathers are somewhat maintained as far as 6-months post-pairing, suggesting females may be capable of maintaining more than one relationship simultaneously. Indeed, patterns of neural activity underlying filial attachment and adult pair bonds was highly overlapping in the present study. It is likely that variation in relationship quality with the father alters neural activity in such a way that makes females more responsive to activation of the OT, AVP, dopamine, and opioid systems.

Significance: We used behavioral, pharmacological, and neuroimaging techniques to address current gaps in knowledge of female social bonding and father-daughter relationships, as well as to elucidate the fundamental behavioral, physiological, and neural correlates of female attachment. OT and AVP were more strongly involved in proximity maintenance behaviors, and these neuropeptide systems were affected by the quality of relationship between fathers and daughters. While relationship quality significantly affected separation distress and stress buffering, it is likely that other neuroendocrine systems underlie separation distress. Results from our neuroimaging study suggest it would be beneficial to examine the role of dopamine and opioid systems in separation distress behaviors in addition to OT and AVP systems. We

established a new method for quantifying relationship quality in titi monkeys and demonstrated the importance of accounting for relationship quality when interpreting responses to social challenges. More broadly, findings from the present studies advance current knowledge of the neurobiological mechanisms foundational to female attachment relationships, and help inform how social disruptions caused by the global pandemic may differently impact humans based on the quality of their social bonds.

CHAPTER 1

Separation distress and stress buffering in juvenile female titi monkeys is mediated by relationship quality with the father

ABSTRACT

Social interactions regulate our behavior and physiology, and strong social bonds can buffer us from stress. Coppery titi monkeys (*Plecturocebus cupreus*) are socially monogamous South American monkeys that display classic attachment behaviors. Infants form selective bonds with their fathers, making them ideal for studying father-daughter bonds. We established a novel method for determining relationship quality in females (n =12). We also investigated how manipulations of oxytocin (OT) and vasopressin (AVP) influenced juvenile attachment. Subjects received acute intranasal treatments of saline, low/medium/high OT, low/high AVP, or OT antagonist (OTA) prior to an acute social separation. General linear mixed-effects model results revealed fathers were significant behavioral and physiological stress buffers for their daughters, as evidenced by fewer distress vocalizations ($p < .001$), less locomotion ($p < .001$), and lower plasma cortisol ($p < .001$). Females vocalized less if they exhibited higher-quality relationships with their fathers as infants ($p = .01$), and this stress buffering effect remained even when the daughter was separated from the father ($p = .001$). While treatments did not alter behaviors, OTA treatments caused the largest rise in plasma cortisol ($p < .001$), suggesting blockade of OT receptors can inhibit fathers' stress-buffering effects. Remarkably, females with higher-quality father-daughter relationships exhibited an overall reduced physiological separation distress response ($p = .04$). Findings from the present study advance current knowledge of the neurobiological mechanisms foundational to female attachment relationships and help inform

how social disruptions may differently impact individuals based on the quality of their social bonds.

INTRODUCTION

Social interactions play a regulatory role in behavioral, psychological, and biological processes (Uchino, 2006). For example, couples in more loving relationships, as measured by the Marital Interaction Scale (Braiker & Kelley, 1979), exhibit a positive correlation between relationship experiences and health habits, which leads to perceptions of better overall health (Markey, Markey, & Gray, 2007). Strong social support may buffer individuals from stress-induced behavioral decisions and related physiological changes, such as cardiovascular reactivity (Uchino, 2006). The connection between social well-being and physical health may be one factor contributing to greater longevity of individuals who are happy with their social relationships (Diener & Chan, 2011).

For group-living animals, including humans, a lack of social connections and poor social relationships have been associated with adverse health outcomes and increased risk of mortality (Holt-Lunstad, Smith, & Layton, 2010). Poor social relationships in particular have been associated with a 29% increased risk for coronary heart disease, and a 32% increased risk for stroke (Valtorta, Kanaan, Gilbody, Ronzi, & Hanratty, 2016). Understanding mechanisms underlying social bonds is especially critical now because the COVID-19 pandemic has detrimentally impacted human social relationships (Evans et al., 2020; Luetke et al., 2020), which may result in adverse health outcomes (Saggioro et al., 2021). Women may be particularly susceptible to psychiatric disorders of social behavior and, compared to men, are more likely to be diagnosed with affective disorders such as major depressive disorder (Weissman & Olfson, 1995). It is possible that social risk factors may contribute to women's increased likelihood of

developing affective disorders (Kuehner, 2017). It is therefore important to study how social bonds may affect health outcomes, particularly in women.

A social bond is defined as a close, selective, enduring, interpersonal relationship that is maintained by physiological and behavioral mechanisms (Bales et al., 2021; Lim & Young, 2006). Bowlby (Bowlby, 1977) argued that humans display attachment behaviors (e.g., seeking proximity with preferred individuals, distress upon involuntary separation from attachment figures) throughout their lives, and Ainsworth (Ainsworth, Blehar, Waters, & Wall, 1978) examined social bonds through the lens of attachment theory. Bonded individuals often display physiological and behavioral synchrony, and the degree of this coordination has previously been shown to be predictive of bonding strength in humans (Feldman, 2007, 2012b, 2012a). Various types of social bonds exist across species, including mother-offspring bonds, pair bonds, and friendships.

A monogamous pair bond is an enduring, selective bond between two adults (Bales et al., 2021), exemplified by a preference for close physical proximity to a specific individual (Carp et al., 2016), aggression towards unfamiliar intruders (Winslow, Hastings, Carter, Harbaugh, & Insel, 1993), distress upon separation from the pair bonded individual (Mendoza & Mason, 1986a), and the ability of partners to buffer each other from stressors (S. Cohen & Wills, 1985; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). Mating also plays a large role in facilitating and maintaining strong affiliative bonds between adults (Lim & Young, 2006; Numan, 2015), but is not necessary for a pair bond (Bales et al., 2021).

Titi monkeys (*Plecturocebus spp.*) are socially monogamous South American monkeys that live in the Amazon basin (Brazil, Bolivia, Peru, Ecuador, and Colombia). In the wild, titi monkeys live in small family groups and raise their offspring together (Fuentes, 1998). Titi

monkeys also display classic attachment behaviors (Fuentes, 1998). For example, titi monkeys maintain proximity with their partners in the lab (Karaskiewicz, Lau, Witczak, Bales, & Dufek, 2021; Rothwell, Carp, Savidge, Mendoza, & Bales, 2020) and in the wild (Dolotovskaya & Heymann, 2020; Dolotovskaya, Walker, & Heymann, 2020; Kinzey & Wright, 1982), potentially as a means of maintaining pair bonds. Titi monkeys also exhibit distress upon separation from their attachment figures and stress buffering by their attachment figures (Hoffman, 1996; Hoffman, Mendoza, Hennessy, & Mason, 1995; Mendoza & Mason, 1986a; Valeggia, 1996). Because titi monkeys exhibit attachments and family structures similar to humans, they are an excellent model species for research topics with human health applications.

Interestingly, the primary attachment figure for titi monkey infants is the father. The fathers spend more time carrying their infants, and when infants are given a choice between interacting with their mothers or fathers, they will choose their fathers (Mendoza & Mason, 1986b). It is likely that infants form a strong attachment to their fathers because fathers spend more time carrying their infants than mothers (Mendoza & Mason, 1986b). Mothers only tolerate holding infants for feeding, and females have been observed immediately soliciting for the male to take the infant once feeding is done and actively avoiding the infant when not nursing in the wild (Dolotovskaya & Heymann, 2020) and in captivity (Karaskiewicz et al., 2021).

Additional evidence for a selective attachment to the father comes from studies on behavioral and physiological responses to separation from and reunion with the father. Titi monkey infants will show behavioral signs of distress upon separation from their fathers, but virtually no response when separated from their mothers if the father is present (Hoffman et al., 1995). For example, infants separated from their attachment figures will show increased vocalizations and locomotion (Mendoza & Mason, 1986b), behaviors which serve as measures of

anxiety in NHPs (Barros & Tomaz, 2002; Hennessy, Mendoza, Mason, & Moberg, 1995). Infants also exhibit a significant rise in plasma cortisol (CORT) levels when separated from the father, and only reunion with the father will attenuate that rise in CORT (Mendoza & Mason, 1986b). Anxiety-like behaviors are also attenuated in the presence of the father, but not the mother.

This selective attachment to the father persists as titi monkeys mature. Even at 27 months of age, female titi monkeys exhibit a sustained elevation in CORT throughout a 5-day separation period, and CORT levels return to baseline only upon reunion with parents (Valeggia, 1996). Daughters as old as 3.5 years of age that are reintroduced to natal groups following one month of social separation are able to rejoin their group and similarly show a reduction in hypothalamic-pituitary-adrenal (HPA)-activity following reunion (Hoffman, 1996). Taken together, these findings suggest that the filial bonds between daughters and their father are long-lasting and continue past puberty (previously estimated to occur between 13-15 months [Valeggia, 1996] but more recently estimated to occur closer to 30 months [Conley et al., 2022] for female titi monkeys).

While titi monkeys consistently exhibit a strong, selective attachment to their fathers, there is a large degree of variability in the degree to which they display behavioral correlates of attachment, such as proximity maintenance, both in the lab (Carp et al., 2016; Hoffman, 1996; Mayeaux, Mason, & Mendoza, 2002) and in the wild (Dolotovskaya, Walker, et al., 2020). For example, titi monkeys have demonstrated a preference for maintaining proximity to their parents in tests conducted at 6, 12, 18, and 24 months of age (Mayeaux et al., 2002), but exhibit a large degree of individual variability within these preference tests. Females between 19 and 48 months of age given the opportunity to interact with unfamiliar conspecifics while in the presence of

their parents still prefer to spend the majority of their time in proximity to their parents, but there are some exceptions (Hoffman, 1996). Even offspring as old as 4 years have been observed huddling in affiliative contact with their parents and family members, demonstrating a maintained affiliative relationship between offspring and family even into early adulthood (Mayeaux et al., 2002). However, around this age some titi monkeys have also been observed emigrating from their natal groups in the wild (Bossuyt, 2002; Norconk, 2011; Souza-Alves, Caselli, Gestich, & Nagy-Reis, 2019; Van Belle, Fernandez-Duque, & Di Fiore, 2016). After being separated from their family and paired with a male for 1.5 years, females still spend more time interacting with their fathers than a stranger male during a preference test, but this is highly variable across individuals (Carp et al., 2016).

The quality of an offspring's relationship with its father may help explain some of this observed variability in displays of attachment-related behaviors throughout development (e.g., proximity maintenance). This has been demonstrated in humans, where attachment relationships in adolescence have been found to influence adult attachments (Dinero, Conger, Shaver, Widaman, & Larsen-Rife, 2008), particularly at the beginning of a new relationship (Dinero, Donnellan, & Hart, 2022). One study has similarly demonstrated an effect of infant attachment on adult relationships in titi monkeys (Savidge & Bales, 2020). In particular, subjects that were more distressed upon separation from their father during an infant social challenge were more affiliative with their partners during the first six months of their first pairing. Subjects that were better buffered from stress by their fathers, as evidenced by decreased anxiety-like behaviors during an infant social challenge, also exhibited lower anxiety-like behaviors in the presence of their partners during a novelty response paradigm (Savidge & Bales, 2020). It is unknown, however, *how* variation in infant and adolescent relationship quality influences later attachment-

related behaviors. It is likely that neurobiological mechanisms important for social bonding and stress buffering underly this variability in attachment behaviors.

Studies of monogamy in prairie voles (*Microtus ochrogaster*) and South American primates, such as owl monkeys (*Aotus spp.*) and titi monkeys (*Plecturocebus spp.*), have demonstrated the importance of oxytocin (OT) and arginine-vasopressin (AVP) for pair bonding (e.g., Bales et al., 2017; Bales, Mason, Catana, Cherry, & Mendoza, 2007; Carter, 1998; Smith, Agmo, Birnie, & French, 2010; Young, Wang, & Insel, 1998). OT and AVP are also involved in human social attachments (Insel & Young, 2001). OT and AVP are both important for approach of novel conspecifics, social recognition, and preferential attachment in males and females (Lim & Young, 2006). Changes in OT and AVP activity during the formation and maintenance of a pair bond have been described extensively in prairie voles and titi monkeys (see Bales et al., 2017).

In addition to OT and AVP, several steroid hormones have also been implicated in the facilitation and maintenance of monogamous pair bonds. One steroid hormone that influences pair bonding is CORT. Female prairie voles require low circulating CORT for the formation of a pair bond, and exhibit high CORT following pair bonding if exposed to a novel male, presumably to prevent her from forming a pair bond with the new male (Devries, Devries, Taymans, Carter, & Marler, 1996; K. A. Young, Gobrogge, Liu, & Wang, 2010). Male prairie voles, on the other hand, require an increase in CORT concentrations to form a pair bond, but will show a decline in CORT when exposed to a novel female regardless of whether or not he was previously pair bonded (K. A. Young et al., 2010). Understanding the interactions between AVP, OT, and HPA-axis activity may help disentangle some of the sex differences in the neurobiology of attachment.

AVP exerts anxiogenic effects and can be co-secreted with corticotropin-releasing factor (CRF) to potentiate the release of adrenocorticotropic hormone (ACTH; Spencer & Deak, 2016). The period of time spent alone immediately following emigration from a natal group may enhance CORT production as well as AVP production, particularly if the period of separation is prolonged (de Goeij et al., 1991), priming an individual to form a monogamous pair bond. Conversely, OT has been demonstrated to indirectly exert an inhibitory tone on the HPA-axis (Carter et al., 2008) through GABA-ergic signaling (Gobrogge & Wang, 2015). Formation of a pair bond or reunion with a mate following a period of separation may increase OT activity, resulting in an increased inhibition of the HPA-axis. This mechanism seems plausible, given the findings in titi monkeys that separation from a mate was hypothesized to only temporarily dysregulate the HPA-axis due to the rapidness of the return to normal circulating CORT levels upon reunion (Mendoza et al., 2000). Human studies have also demonstrated that intranasal OT in conjunction with social support can reduce salivary CORT levels during a Trier Social Stress Test in men (Heinrichs et al., 2003). OT may therefore be a primary pathway regulating the stress-buffering effects of social support on other physiological systems.

Indeed, administration of OT has been demonstrated to inhibit CORT activity in both male and female prairie voles (Carter, 1998). However, males and females exhibit opposite dose-response curves with regards to the proportion of time they spend in contact with their partners versus a stranger when treated intranasally with OT (Carter et al., 2008). Males spend more time in contact with their partners than strangers when treated with a low (1 mg/kg) or high (4 mg/kg) dose of OT, but spend equal time with strangers and partners when given a medium dose (2 mg/kg). Females, on the other hand, spend more time in contact with their partner when treated with the medium dose, and equal time with the stranger and partner when given the low or high

dose of OT (Carter et al., 2008). Further investigation of the interactions between OT, AVP, and CORT may help us better understand the cause of sexual dimorphism in attachment behavior. Females in general have been greatly understudied, and so it is particularly important to investigate the neurobiological mechanism underlying female attachment.

The roles of OT and AVP in titi monkey daughters' responses to separation and reunion with their father are unclear. One way to investigate the role neuropeptides play in attachment-related behaviors is to study the effects of exogenous manipulations of OT and AVP during a social challenge. One challenge with manipulating the OT and AVP system is that these molecules are lipophobic and do not readily cross the blood-brain-barrier. However, the use of intranasal treatments has proven to exert central effects, altering neuronal activity and cognition in humans (for a meta-analysis, see: Bakermans-Kranenburg & Van Ijzendoorn, 2013) and non-human animal models (for a review, see: Freeman & Young, 2016). It is hypothesized that intranasal OT reaches the brain via the trigeminal or olfactory nerve (Veening & Olivier, 2013). A single dose of intranasally administered OT has been shown to be elevated in CSF starting at 15 minutes and lasting from 30-120 minutes (Freeman et al., 2016).

The effects of intranasal OT may be long-lasting due to the feedforward effect of OT where an increase in OT may lead to a further increase in OT (like it does during birth; Bakermans-Kranenburg & Van Ijzendoorn, 2013). Larger doses of OT, however, may have the opposite effect if OT binds to AVP receptors. While OT generally has anxiolytic effects, AVP has a synergistic relationship with CRH and produces anxiogenic effects (Gobrogge & Wang, 2015). OT and AVP have very similar structures and can bind to each other's receptors (Gimpl & Fahrenholz, 2001). Therefore, if one were to explore the use of OT to reduce anxiety, it would

be essential to administer a dosage that was large enough to produce effects but not so large that OT would also activate AVP receptors.

In titi monkeys, as in the prairie voles, chronic treatment with a low dose (0.8 IU/kg OT) facilitates partner preference in adult males but not females (Arias del Razo et al., 2022). Interestingly, chronic intranasal OT increased time spent in proximity with their parents for females but not males (Arias del Razo et al., 2020). Therefore, to better understand the effects of exogenous manipulations of OT on female attachment behaviors, it is important to administer different dosages to investigate a potential dose-response curve.

The effects of acute intranasal AVP treatments have also been investigated in male titi monkeys (Jarcho, Mendoza, Mason, Yang, & Bales, 2011). This previous study used both a 40 IU/kg and 80 IU/kg dose and found that the higher dose was effective in altering males' behaviors towards their mates. Jarcho and colleagues (Jarcho et al., 2011) used a sequential preference test, presenting males with either their partner, an unfamiliar female, or an empty box. When treated with saline, males spent more time interacting with the stranger females. At the low dose of AVP, males did not show a preference for either female; however, the high dose of AVP resulted in a significant preference for interacting with their partners during the sequential preference test. Given the sex differences observed in OT manipulation studies, it is likely that females may respond differently to manipulations of AVP.

In addition to understanding the effects of exogenous agonists, it is essential to study the effects of antagonists that block receptor activity. There is an oxytocin receptor antagonist (OTA) that is highly selective for OTR in primates and has been used successfully for behavioral pharmacology experiments in marmosets (Cavanaugh, Mustoe, Taylor, & French, 2014). This antagonist has also been effective in exerting central effects in rhesus macaques when given

peripherally through intravenous administration (Boccia, Goursaud, Bachevalier, Anderson, & Pedersen, 2007). Assays of brain tissue samples 60 minutes post-administration confirmed presence of the compound in the hypothalamus, septum, orbitofrontal cortex, amygdala, and hippocampus. Adult females treated with the OTA exhibited reduced or eliminated interest in infants and sexual behavior (Boccia et al., 2007).

While Cavanaugh and colleagues (Cavanaugh et al., 2014) gave their dose orally and Boccia and colleagues (Boccia et al., 2007) administered their treatment intravenously, Smith and colleagues (A. L. Smith et al., 2017) tracked presence of a similar OTA compound in the plasma and CSF of male rhesus macaques following intranasal administration of the antagonist. The average low dose administered was 205 μg while the average high dose was 7.69 mg. For the high dose, the drug was detectable in the CSF 60- and 120-minutes post-administration.

Given the variability in responses to manipulations of the OT system in males and females and the lack of studies on the effects of manipulations of the AVP system in female titi monkeys, it is essential to investigate how exogenous manipulations of these neuropeptide systems impact female attachment-related behaviors. Given the large degree of variability in titi monkeys' responses to social challenges in previous laboratory studies, it is important to investigate how relationship quality affects responses to social challenges. Our main objectives were to understand how relationship quality and manipulations of the OT and AVP systems impact the behavioral and physiological correlates of separation distress and stress buffering.

We had several predictions regarding the impact of relationship quality and neuropeptide manipulations on separation distress and stress buffering:

1) We predicted that a higher-quality relationship with the father, as evidenced by greater distress upon separation and greater proximity maintenance as infants and

juveniles, would enhance separation distress and stress buffering. For example, we predicted females with a higher-quality relationship with their fathers would exhibit a greater rise in plasma CORT when separated from their father compared to females with a lower-quality relationship. However, we also predicted that females with a high-quality relationship would exhibit lower CORT levels when tested with their father compared to females with lower-quality relationships, because their fathers may serve as a better stress buffer during this social challenge.

2) Based on previous research using prairie voles (Carter et al., 2008) and findings of a study investigating the effects of chronic intranasal OT administration with juvenile titi monkeys (Arias del Razo et al., 2020), we predicted females would require a higher dose of OT than males to exhibit alterations in their separation distress response. Specifically, we predicted the low dose of OT might not be strong enough to buffer females from separation distress.
3) We predicted the medium dose of OT would result in a blunted stress response (demonstrated by lower plasma CORT concentrations, fewer vocalizations, and less time spent pacing during an acute social challenge).
4) The high dose of OT was predicted to potentially bind to AVPr1a, which could elicit a greater stress response than the low/medium doses of OT and saline if activation of AVPr1a activated the HPA-axis and overrode the anxiolytic effects of OTR activation.
5) AVP typically exerts anxiogenic effects; therefore, we predicted the low dose of AVP would result in a high separation distress response.

6) AVP does have a high affinity for OTR; therefore, if the large dose resulted in activation of OTR, we predicted HPA-axis activity may be dampened.
7) OTA was expected to exert anxiogenic effects, increasing physiological and behavioral markers of stress.

METHODS

Subjects & Housing

Subjects were 12 juvenile female titi monkeys (ages 14-18 months) and their fathers (N = 12 juvenile females; N = 12 fathers). The juvenile period represents the peri-pubertal stage.

Subjects were housed at the California National Primate Research Center and lived in their natal groups with their parents and any older siblings that were still in the natal group when the subject turned 14 months of age. Families were housed in a 1.2 m x 1.2 m x 2.1 m stainless steel cage or a 1.2 m x 1.2 m x 1.8 m with four horizontal perches, a food bowl, and two water dispensers. They were fed twice daily with Purina New World monkey chow, rice cereal, carrots, apples, and bananas. They were kept on a 12 hour light, 12 hour dark cycle, with lights coming on at 0600 hours and turning off at 1800 hours. The room temperature was maintained at approximately 21°C. For additional information regarding animal husbandry, please see Tardif and colleagues (Tardif et al., 2006) and Mendoza and Mason (Mendoza & Mason, 1986a). This study was approved by the IACUC of the University of California, Davis; and complied with legal requirements of the United States.

Social Separation Data Collection

The females for our juvenile tests began testing at 14 months of age and completed approximately 15 weeks later. Subjects were habituated to being captured in a transport cage, handled, and intranasally treated for five weeks prior to the start of testing. We spent one week

training subjects to enter transport cages in their home-cage, one week habituating them to being handled, one week habituating them to being restrained in preparation for intranasal treatment, one week being restrained while we touched their nostrils with a pipette tip, and one week administering 45 ul of water in each nostril while restrained. At the start of testing, all subjects went through one baseline blood draw at 14 months of age. We collected an unsedated 0.5 ml femoral blood sample using 1-cc syringes pretreated with heparin at 1130 hours. Subjects were captured in a transport cage, hand captured, and manually restrained for sample collection. All blood samples were collected within 5 minutes of entry into the subject's home-cage. Following blood collection samples were immediately placed on ice, centrifuged at 1,610 x *g* at 4°C, and the plasma was extracted and stored at -80°C until assay.

Starting one week later, subjects underwent 14 separation tests (seven in which mothers were removed, leaving the females with their fathers, and seven in which both parents were removed). At the start of the test, starting at 1030 hours, subjects were captured in a transport cage, manually restrained, and received an intranasal dose of one of seven treatments (saline, 0.8 IU/kg OT, 8.0 IU/kg OT, 80.0 IU/kg OT, 40 IU/kg AVP, 80 IU/kg AVP, or 10 mg/kg OTA). Given the variable responses to chronic intranasal OT treatments of 0.8 IU/kg OT (Arias del Razo et al., 2020, 2022), we chose 0.8 IU/kg OT as our lowest dose and chose two higher doses (8.0 IU/kg and 80.0 IU/kg OT) to investigate a potential dose-response curve. These higher doses have been used in rodents without adverse effects (Bales et al., 2013). We selected 40 IU/kg and 80 IU/kg for AVP treatments because those were previously used in male titi monkeys (Jarcho et al., 2011). We chose 10 mg/kg of OTA based on findings from a previous study that used the same OTA intranasally (A. L. Smith et al., 2017) to ensure effectiveness of the treatment while minimizing the risk of adverse side effects. Separation conditions and treatment order were

counter-balanced to control for effects of time. Tests were spaced one week apart to ensure drug washout. All treatments were expected to be completely out of the subject's system after 48-hours, and previous studies have tested subjects every other day, so we did not anticipate any carry-over treatment effects one week later (Cavanaugh et al., 2014; Jarcho et al., 2011). We also wanted to keep the developmental period relatively consistent between and within subjects while minimizing stress on the subjects and their families.

Females were returned to their families for a 30-minute uptake period immediately following dosing. Following the 30-minute uptake period, at approximately 1100 hour, we either removed just the mother (stress buffered condition) or both the mother and father (separation distress condition). Only one of our 12 subjects had an older sibling, and that sibling was always removed after the uptake period. Family members were taken to a separate building so subjects did not have visual, auditory, or olfactory access to them. During the separation period, we live scored vocalizations using Behavior Tracker (www.behaviortracker.com) and recorded vocalizations using a Marantz PMD 660 solid-state digital flash recorder and a RODE NTG-2 directional condenser microphone. We used previously defined classifications of titi monkey vocalizations (Clink, Lau, & Bales, 2019; Lau, Clink, & Bales, 2020; Robinson, 1979) as well as spectral and temporal analysis to make quantitative definitions for all the vocalizations that we identified by ear using Raven Pro 1.5 Sound Analysis Software (Bioacoustics Research Program 2014, Ithaca, NY). The actual scoring of the vocalizations was done live after the four types of vocalizations had been identified (Table 1). For analyses, we combined three of the call types (trills, whistles, peeps) because all are likely to signal separation distress or used as a means to communicate with their attachment figure (Hoffman et al., 1995; Larke, Toubiana, Lindsay, Mendoza, & Bales, 2017; Symmes & Biben, 1985). We analyzed long calls separately because

they are distinctly different from the other three call types and thought to be used by titi monkeys for several purposes, including coordinated territorial defense (Marshall & Marshall, 1976), but this is still under investigation (Marshall-Ball, Mann, & Slater, 2006). All tests were video recorded and later scored using Behavior Tracker (www.behaviortracker.com) for locomotion, which serves as a behavioral correlate for anxiety (Capitanio, Blozis, Snarr, & Steward, 2017; Capitanio, Kyes, & Fairbanks, 2006; Ragen, Maninger, Mendoza, & Bales, 2015). Locomotion was defined as moving one body length or more and being in motion for at least one second (Table 1).

At the completion of the 30-minute separation period, around 1130 hours, we collected an unsedated 0.5 ml femoral blood sample using the methods described above. Blood samples were placed on ice immediately, centrifuged at 1,610 x *g* at 4ºC, and the plasma extracted and stored at -80ºC until assay. Families were reunited and we recorded parent-offspring interactions for 15 minutes.

Throughout the duration of testing, urine samples were collected three times per week to track subjects' reproductive status. It was not expected that females would be cycling at this age while still in their natal group (Conley et al., 2022; Valeggia, 1996).

Plasma Cortisol Assay

We used an enzyme immunoassay validated for titi monkeys to estimate plasma CORT concentrations (for detailed assay methods, see Witczak et al., 2021). Briefly, plates (Thermo Nunc Maxisorp 442404P) were coated at a concentration of 1:20,000 with antisera R4866 (produced by UC Davis Endocrinology Laboratory) in 50 ul coating buffer (0.05M carbonate, pH 9.6) per well, overnight, and stored at 4º C until use. Plasma was not extracted, but was diluted 1:1000 in EIA buffer (0.1M phosphate buffer, pH 7.0, 0.1% bovine serum albumin), in

microtubes. Results were analyzed using Softmax software with a 4-place fit using concentration vs. a percent binding calculation of the standard curve. A total of six plates were assayed, with intra-assay CVs of 4.5%, 13.2%, 10.0%, 9.0%, 0.1%, and 8.1%, with an inter-assay CV of 19.2%.

Quantification of Relationship Quality

We have access to several measures of infant and juvenile relationship quality in our captive titi monkey colony. For the present study, we quantified several indicators of separation distress and proximity maintenance for our 12 subjects (Table 2). Measures were collected both as scan samples and as responses to social challenges when subjects were infants and juveniles. For the data analysis, all measures of relationship quality were centered about the mean value for our 12 subjects. This allowed us to determine how variation in relationship quality influences various outcomes in our testing paradigm. For example, we could examine how females that spend a greater proportion of time in proximity to their fathers as infants differ from females that spend comparatively less time maintaining proximity with their fathers. Below are details regarding data collection for each measure of relationship quality:

IOF Locomotion

All infant titi monkeys at the California National Primate Research Center undergo infant open field (IOF) tests when they are four months of age. This test measures responses to a novel environment and reactions to the presence of family members during a stressful experience. Subjects are placed in the center of a one meter by one meter arena with a six-by-six square floor grid. One wall included a wire mesh grate that allowed auditory, visual, and olfactory access to a stimulus animal that was placed in a transport box on the other side of the grate. During this testing, subjects experienced four five-minute trials, each with a different stimulus on the other

side of the grate (their father, their mother, their oldest sibling [if they had any], and an empty box). For a more detailed description of the infant open field methods, see Larke and colleagues (Larke et al., 2017) and Savidge and Bales (Savidge & Bales, 2020). During testing, we count the number of lines that the infant crosses within each five-minute trial (defined as two or more limbs crossing a line; Larke et al., 2017). For the present study, we calculated the number of line crosses for each subject during the empty box condition and the father condition. We then calculated the percent change in line crosses from the father condition to the empty box condition ([{line crossing when alone – line crossing with father} / line crossing with father] * 100). We interpreted a greater increase in line crossing to indicate heightened distress upon separation, which may be an indicator of a higher-quality relationship with the father.

IOF Vocalization

During IOF testing we also score infant vocalizations. During this test we typically only hear infants make trill calls (as defined by Clink et al., 2019), but if they made any other separation distress calls, we combined those with the number of trills scored to create a total vocalization score. We then calculated the percent change in vocalizations from the father condition to the empty box condition. A greater increase in vocalizations was interpreted as an enhanced separation distress response, which is assumed to be an additional indicator of a higher-quality relationship with the father.

Juvenile Locomotion

We quantified measures of juvenile relationship quality using behaviors scored during the saline condition of our social separation testing. We quantified the duration of time females spent locomoting when females were tested alone (separation distress condition) and when they were tested with their father (stress buffering conditions), and calculated the percent change in

locomotion from the stress buffering to the separation distress condition. We interpreted a greater increase in locomotion as a heightened separation distress response, which may be indicative of a higher-quality relationship with the father.

Juvenile Vocalization

We also quantified percent change in separation distress vocalization frequency from the stress buffering to the separation distress conditions of the social separation test when females were treated with saline. The values for this measure were much larger than the values for all other relationship quality variables; therefore, we rescaled this measure by dividing by 10 to make this variable on a more similar scale to our other predictors. A greater increase in vocalizations from the stress buffered to the separation distress condition represented an elevated stress response, which we interpreted to be indicative of a higher-quality relationship with the father.

Juvenile Proximity

We also quantified measures of juvenile proximity maintenance using video recordings from our social separation testing. Females were reunited with their families in their home-cage immediately following blood sample collection, and their interactions were recorded for 15 minutes. For this measure, we only looked at behaviors during the reunion period following the saline condition of the separation distress condition (when females were left alone in the home-cage for 30 minutes). We calculated the duration of time that females spent in proximity, contact, or tail-twining with their fathers during this 15-minute reunion period (for detailed definitions of these behaviors, see Karaskiewicz et al., 2021). We calculated the percentage of time females were in social proximity with their fathers as the total time in social proximity/contact/tail-twining out of the entire 15-minute reunion period ([duration of time in contact/proximity/tail-

twining / 600 seconds] * 100). We interpreted a greater percentage of time maintaining social proximity to the father as aa higher-quality relationship with the father.

IOF Proximity

In addition to quantifying measures of separation distress during IOF testing, we were also able to quantify measures of infant proximity maintenance. We used the same definition of grate proximity that Larke and colleagues (Larke et al., 2017) used to quantify percent time infants spent in close proximity to the grate in the IOF arena. We calculated the percentage of time subjects spent in proximity to the grate during the father condition by dividing the amount of time in proximity to the father by 300 seconds. We assumed greater increase in percentage of time spent in close proximity to the father during testing indicated a higher-quality relationship with the father.

IOF Grate

We also quantified the amount of time the females spent physically touching the grate in the IOF arena. Females had to have at least one hand or foot on the grate to be considered touching the grate (touching the grate with the tail or a different part of the body did not count as grate touching in this test to mirror how grate touching is scored in preference tests [Carp et al., 2016]). We calculated the percentage of time spent touching the grate during the father condition of IOF testing as an additional measure of infant proximity maintenance. We also interpreted a greater percentage of time spent touching the grate as indicating a higher-quality relationship with the father.

Infant Proximity

From the day an infant is born until they are nine months old, we conduct spot checks every two hours, five days per week, to record who is carrying the infant (mother, father, or

sibling) or whether the infant is out of contact with any family member and independently moving about the home-cage (Karaskiewicz et al., 2021). The percentage of time spent being carried by a family member out of all checks recorded can give us a measure of the quality of parental care an infant receives. For the present study, we quantified the percentage of time females were carried by their father out of all scan samples collected for that subject. We assume greater percentage of time being carried by the father early in life could be a predictor of a better father-daughter relationship in this species, and possibly result in a higher-quality relationship with the father.

Parent Affiliation

In addition to the infant scan samples, as soon as a titi monkey pair is established, our lab conducts pair-mate scan samples every two hours to record where pair-mates are in relation to each other (Karaskiewicz et al., 2021). The four behaviors recorded on our check sheets are proximity, contact, tail-twining, and nothing. Check sheets are completed five days per week for the entire time that a pair is together. This measure of pair-mate affiliation gives us an indication of the overall level of affiliation that the infant observes in the natal group. In human studies, parents' relationship quality has been found to be a predictor of children's future romantic relationship quality (Gager, Yabiku, & Linver, 2016); therefore, this measure may help us better understand variation in titi monkey attachment. We assume greater percentage of time in affiliative contact is an indicator of higher quality relationships in this species, and may set the foundation for infants to form higher-quality relationships. For the present study, we quantified the percentage of time parents were in proximity, contact, or tail-twining out of all scan samples collected on the parents during the first 14 months of our subjects' lives.

Data Analysis

All analyses were conducted in R Statistical Software (version 4.0.3, R Core Development Team, 2020). For all outcome measures, we performed a Shapiro Wilk test of normality and transformed non-normally distributed variables as necessary (Royston, 1983). All tests were two-tailed and the significance threshold was set at .05.

We first identified which of the nine relationship quality variables (*IOF Locomotion, IOF Vocalization, Juvenile Vocalization, Juvenile Locomotion, Juvenile Proximity, Infant Proximity, IOF Proximity, IOF Grate, Parent Affiliation;* Table 3) best explained variance in our four outcome variables (distress vocalizations [trills, whistles, and peeps combined], long calls, locomotion, plasma CORT). To identify best-fitting relationship quality variables, we ran stepwise regression using the leaps package (Lumley, 2020). This method allowed us to iteratively add and remove variables in the predictive model to identify which subset of variables resulted in the model with the lowest prediction error (Bruce & Bruce, 2017; Gareth, Witten, Hastie, & Tibshirani, 2014). To simplify the stepwise regression models, we first ran separate stepwise regression models for separation distress (*IOF Locomotion, IOF Vocalization, Juvenile Locomotion, Juvenile Vocalization*) and proximity maintenance (*Juvenile Proximity, IOF Proximity, IOF Grate, Infant Proximity, Parent Affiliation*) variables. Once we had identified the top separation distress and proximity maintenance variables, we ran a final stepwise regression model that just included those top variables from each category (separation distress and proximity maintenance). The combination of relationship quality variables that was identified as producing a model with the lowest prediction error was then used in our mixed-effects models.

For separation distress vocalizations, locomotion, and plasma cortisol, we ran general linear mixed-effects models (LMM) using the lmerTest package (Kuznetsova, Brockhoff, & Christensen, 2017), with animal identity as a random effect to account for repeated measures.

Our models were specified as: $y_{ij} = \beta_0 + \beta_1 x^{(1)}_{ij} + \cdots + \beta_m x^{(m)}_{ij} + \eta_{i0} + e_{ij}$. In this model, y_{ij} denotes the *j*th observation taken of individual *i* for outcome variable *y.* Individual covariates included in the model are represented by *x* (covariates 1-m), which have fixed effects common to all individuals in the population (β). This model also includes a random effect of ID that is specific to each individual (η_{i0}). Residual errors are represented by e_{ij}. In our full model, fixed effects included *Treatment*, *Test Number*, *Condition* (separation, buffering), relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Condition* and each relationship quality variable. To determine the best-fitting model, we used backwards selection to remove any non-significant fixed effects (Bentler & Mooijaart, 1989). We used a log likelihood ratio test to compare model fit to determine whether removing any non-significant fixed effects resulted in a better fitting model (Vuong, 1989; Supplementary Table 1). We then performed a log likelihood ratio test to compare the fit of our best model to that of the null model where we removed all main effects (Vuong, 1989; West, Taylor, & Wu, 2012). The resulting final model, determined using a model comparison based on the likelihood of the model to the data, is the only one in which we evaluated the significance of the remaining parameters. Therefore, for each outcome variable, we had one final model where we interpreted significance level. The one final model represented the most likely hypothesized relationship between parameters given the data. Because we only evaluated parameter significance in one model per outcome, post hoc corrections were not necessary (Vuong, 1989; West et al., 2012). For all significant predictors we also calculated Cohen's f^2 as a measure of effect size (Selya et al., 2012; Snijders & Bosker, 1994). Based on Cohen's (J. Cohen, 1988) guidelines, $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively. We also had strong *a priori* hypotheses regarding how relationship quality variables and treatments would impact our

outcome variables. We predicted that a higher-quality relationship with the father, as evidenced by greater distress upon separation and proximity maintenance, would result in greater distress upon separation in the alone condition, but reduced markers of distress when females are tested in the presence of their father. In general, we also predicted agonism of OT receptors would reduce correlates of separation distress, antagonism of OT receptors and agonism of AVP receptors would enhance separation distress, and very high doses of OT and AVP agonists may result in opposite effects if they bind to each other's receptors and impact HPA-axis activation.

For long calls, because there was a large number of tests where females did not long call at all, we ran zero-inflated models with a Poisson distribution using the GLMMadaptive package (Rizopoulos, 2022). This allowed us to separately model the absence of long calls and explain variability when females did long call. In our fullest model, we included subject ID as a random effect for both the response and zero-part of the model. We also included the main effects of *Treatment*, *Test Number*, *Condition* (separation, buffering), relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Condition* and each relationship quality variable. Similar to our methods for our LMM analyses, we used backwards selection to remove non-significant fixed effects and compared model fit using a log likelihood ratio test. The best-fitting model was compared to a null model, and parameter significance was only interpreted in that final model.

RESULTS

Separation Distress Vocalizations

Because the residuals for separation distress vocalizations were non-normally distributed, we log-transformed the data using base e ($\log_e$ transformation). Based on stepwise regression analyses, the variables *Treatment, Juvenile Vocalization, Juvenile Proximity, Infant Proximity,*

IOF Grate, Juvenile Locomotion, and *IOF Proximity* significantly predicted separation distress vocalizations duration during separation testing. We then compared a series of general linear mixed-effects models that included these predictors as well as ID as a random, repeated measure and *Condition* (father or alone) as additional predictors of separation distress vocalization frequency. Our identified predictors from the stepwise regression analyses were allowed to interact with *Condition* in our full model. Based on a log likelihood ratio test, the model that included the main effects of *Condition, Infant Proximity, Juvenile Locomotion, IOF Proximity*, plus the interaction between *Condition* and *Juvenile Locomotion* and *Condition* and *IOF Proximity* fit best (Table 4a). This model also fit better than our null model.

The R^2 for this model is 0.6753. In general, females vocalize less when with their fathers (Mean = 88.01, SD = 182.18) compared to when they are tested alone (Mean = 462.27, SD = 323.55).

Based on the results of this best-fitting model, we have evidence to suggest that females vocalize significantly less with their fathers (β= -2.184, SE = 0.014, t = -15.958, p < .001, f^2 = 1.361; Figure 1a), and less if they spent more time being carried by their fathers as infants (β = -0.061, SE = 0.023, t = -2.709, p = .027, f^2 = 0.159; Figure 1b). There was a significant interaction effect between *Condition* and *Juvenile Locomotion* (β = 0.002, SE = 0.0004, t = 3.729, p < .001, f^2 = 0.066; Figure 1c), suggesting that females vocalize more when they are with their father if they show a higher increase in locomotion from the father to the alone condition when treated with saline. However, they vocalize slightly less in the alone condition if they exhibited a higher increase in locomotion in the saline condition. We found a significant interaction between *Condition* and *IOF Proximity* (β = 0.024, SE = 0.005, t = 4.386, p < .001, f^2 = 0.092; Figure 1d)

which suggested that females that spent more time in proximity to their fathers during IOF testing vocalize more during the father condition but less in the alone condition.

Long Call Vocalizations

Based on stepwise regression analyses, the variables *Treatment, IOF Vocalization, Juvenile Vocalization, Juvenile Proximity*, and *IOF Grate* significantly predicted long call frequency during separation testing. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution that included these predictors as well as ID as a random, repeated measure and *Condition* (father or alone) as additional predictors of long call frequency. These predictors were also allowed to interact with condition in our full model. Based on a log likelihood ratio test, the model that allowed predictors to enter the model differently for non-zero and zero responses fit best. For our best-fitting model, the non-zero responses were predicted by *Treatment, Condition, IOF Vocalization, Juvenile Vocalization, Juvenile Proximity*, the interaction between *Treatment* and *Condition*, the interaction between *Condition* and *IOF Vocalization*, and the interaction between *Condition* and *Juvenile Proximity*. The zero responses were best explained by *Treatment, Condition, IOF Vocalization*, the interaction between *Treatment* and *Condition*, and the interaction between *Condition* and *IOF Vocalization* (Table 4b). This model also fit better than our null model.

Treatments did significantly impact long calling behavior. When treated with saline, females long called an average of 6.67 (SD = 8.62) times. Long call frequency was reduced in females, regardless of whether they were tested with their fathers or alone, when they were treated with Low AVP (Mean = 4.00, SD = 4.73) and OTA (Mean = 4.29, SD = 5.98). Compared to when treated with saline, females long call less when treated with any of the OT treatments

(Low OT Mean = 5.75, SD = 7.62; Medium OT Mean = 5.46, SD = 8.85; High OT Mean = 4.88; SD = 8.81) and High AVP (Mean = 3.62, SD = 5.69).

Based on the results of this best-fitting model, we have evidence to suggest that when females long call, they call less frequently if treated with Low AVP (β = -0.511, SE = 0.171, Z = -3.000, p = .003; Figure 2a) or OTA (β = -0.415, SE = 0.160, Z = -2.590, p = .010; Figure 2a), regardless of condition. However, when females long call, they will long call less in the father condition when treated with High AVP (β = -0.592, SE = 0.290, Z = -2.040, p = .041; Figure 2a), High OT (β = -0.683, SE = 0.282, Z = -2.418, p = .016; Figure 2a), Low OT (β = -0.902, SE = 0.258, Z = -3.501, p < .001; Figure 2a), and Medium OT (β = -1.019, SE = 0.261, Z = -3.900, p < .001; Figure 2a) compared to saline. The interaction between treatment and condition also explains when females do not long call. Specifically, females treated with Low OT (β = -3.476, SE = 1.693, Z = -2.054, p = .040) and Medium OT (β = -2.984, SE = 1.513, Z = -1.973, p = .049) are more likely not to long call in the father condition compared to females treated with saline.

Females that vocalize more in general when separated from their father also long call more (β = 0.001, SE = 0.0003, Z = 5.240, p < .001; Figure 2b). We found two significant interaction effects that explain variability in when females long call. When females long call, females that spend a greater amount of time in proximity to their fathers during the reunion period long call more in the alone condition and less in the father condition (β = -0.020, SE = 0.004, Z = -5.153, p < .001; Figure 2c). When females long call, if they exhibited a greater increase in vocalizations during IOF testing when alone compared to when with the father, they long call more in both the alone and father conditions (β = -0.003, SE = 0.001, Z = -2.718, p = .007; Figure 2d). This interaction between condition and IOF vocalizations also explained when females did not long call at all (β = -0.019, SE = 0.007, Z = -2.594, p = .009).

Locomotion Duration

The residuals for locomotion duration were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Test Number, Infant Proximity,* and *Infant Locomotion* significantly predicted locomotion duration during separation testing. We then compared a series of general linear mixed-effects models that included these predictors as well as ID as a random, repeated measure and *Condition* (father or alone) as additional predictors of locomotion duration. *Infant Proximity* and *IOF Locomotion* were allowed to interact with *Condition* in our full model. Based on a log likelihood ratio test, the model that included the main effects of *Test Number, Condition, Infant Proximity, IOF Locomotion,* plus the interaction between *Condition* and *Infant Proximity* fit best (Table 4c). This model also fit better than our null model.

The R^2 for this model is 0.3502. In general, females locomoted less when tested with their fathers (Mean = 194.06 seconds, SD = 118.24 seconds) compared to when alone (Mean = 299.86 seconds, SD = 157.58 seconds). Based on the results of this best-fitting model, we have evidence to suggest that females locomote significantly less with their fathers (β = -3.307, SE = 0.614, t = -5.390, $p < .001$, $f^2 = 0.480$; Figure 3a), but more as test number increases from one to 14 (β = 0.359, SE = 0.076, t = 4.701, $p < .001$, $f^2 = 0.129$; Figure 3b). Females also locomote more during testing if they showed a greater increase in line crossing behavior from the alone to father condition during infant open field testing (β = 0.007, SE = 0.002, t = 3.295, p = .009, f^2 = 0.082; Figure 3c). There was a significant interaction between *Condition* and *Infant Proximity* (β = -0.405, SE = 0.094, t = -4.288, $p < .001$, $f^2 = 0.108$; Figure 3d). When females are in the alone condition, they will locomote more if they spend a lot of time being carried by their fathers as infants; however, in the father condition there is a slight negative relation between locomotion

duration and *Infant Proximity*, suggesting females in the father condition may locomote less if they are carried more as infants.

Plasma Cortisol

The residuals for plasma CORT concentration were normally distributed following a $\log_e$-transformation. Based on stepwise regression analyses, the variables that significantly predict cortisol during separation testing include *Treatment, IOF Vocalization, Juvenile Vocalization, Juvenile Locomotion, IOF Grate, Juvenile Proximity,* and *IOF Proximity.* We then compared a series of general linear mixed-effects models that included these predictors as well as ID as a random, repeated measure and *Condition* (father or alone) as additional predictors of cortisol concentration. All relationship quality variables were allowed to interact with *Condition* in our full model. Based on a log likelihood ratio test, the model that included the main effects of *Treatment, Condition, Juvenile Locomotion, IOF Grate,* and *Juvenile Proximity* fit best (Table 4d). This model also fit better than our null model.

The R^2 for this model is 0.5894. Based on the results of this best-fitting model, we have evidence to suggest that females exhibit a smaller rise in plasma cortisol when with their fathers compared to the alone condition ($\beta = -0.32$, SE = 0.044, $t = -7.264$, $p < .001$, $f^2 = 0.215$; Figure 4a). Females treated with OTA exhibited the largest rise in plasma cortisol (Mean = 657.24 ng CORT/ml plasma, SD = 243.04 ng CORT/ml plasma) compared to the saline condition (482.63 ng CORT/ml plasma, SD = 171.38 ng CORT/ml plasma). Based on the results of our best-fitting model, females treated with the oxytocin antagonist (OTA) exhibited the largest rise in plasma cortisol, regardless of whether females were with their fathers or alone ($\beta = 0.286$, SE = 0.082, $t = 3.472$, $p < .001$, $f^2 = 0.079$; Figure 4b). Females also exhibited a higher rise in cortisol if they spent more time in proximity to their father during IOF testing ($\beta = 0.008$, SE = 0.003, $t = 2.598$,

p = .036, f^2 = 0.224), but a lower rise in cortisol if they spent more time touching the grate during IOF testing (β = -0.010, SE = 0.004, t = -2.478, p = .042, f^2 = 0.203; Figure 4c).

DISCUSSION

We found support for several of our predictions, particularly our prediction that the quality of a daughter's relationship with her father would enhance stress buffering. Interestingly, each outcome variable tested (separation distress vocalizations, long call vocalizations, locomotion, and plasma CORT) revealed slight differences with regards to which relationship quality predictor (Table 2) significantly explained variability in responses. With regards to separation distress vocalizations, females vocalize less when with their fathers compared to when they are tested alone. If they spent more time being carried as infants, females vocalized even less, suggesting fathers do have a buffering effect with regards to this type of anxiety-like behavior. Interestingly, females that exhibit a greater amount of distress upon separation (via greater increase in *Juvenile Vocalizations* as well as more time in proximity to the father during IOF testing) vocalize more when with their father but less when alone. When females are tested with their fathers, their fathers are experiencing a separation from their partner, and tend to vocalize a fair amount. Females in this father condition that were themselves more distressed upon separation from their father appear to somewhat mirror their fathers' behavior and vocalize a lot themselves. This behavior is similar to the synchronization of behaviors observed between titi monkey pair-mates during an acute social intruder challenge (Mercier, Witczak, & Bales, 2020). While we did not score fathers' vocalizations during testing, it would be interesting to quantify the degree of synchronization in vocalization behavior between fathers and daughters in future studies. Despite females with higher-quality relationships with their fathers vocalizing more when tested with their fathers, when these same females are tested alone, they vocalize

less, suggesting the fathers may have some lingering buffering effects on their daughters if they exhibit these behavioral indicators of higher-quality relationships. These findings are similar to those found in humans that have demonstrated that strong, healthy attachment relationships are able to buffer children and adults from various stressors (for a review, see Ditzen & Heinrichs, 2014). Therefore, the mechanisms driving social buffering in titi monkeys may be similar in humans.

We had predicted that agonism of the OT system would decrease separation distress vocalizations while agonism of the AVP system would increase vocalizations; however, none of our treatments significantly explained separation distress vocalizations. It is possible that other neuropeptide systems are more relevant for mediating certain distress behaviors. For example, a recent review of neurobiological mechanisms related to separation anxiety disorder in humans highlighted the roles of the serotonin, opioid, dopamine, and GABA systems (Schiele, Bandelow, Baldwin, Pini, & Domschke, 2020). The opioid system in particular is important in mother-infant bonds, and lower levels of endogenous opioids are associated with heightened separation anxiety (Preter & Klein, 2014) while exogenous opioid agonists reduce separation distress responses during social isolation (Herman & Panksepp, 1978). Future studies should repeat these social separation paradigms using exogenous opioid manipulations to determine whether titi monkeys exhibit similar stress buffering effects.

While manipulations of OT and AVP did not significantly explain separation distress vocalizations, they did affect long calling vocalizations. Because several females never or rarely ever long called, we used a zero-inflated model to explain when females did and did not long call. Long call frequency was reduced in females, regardless of whether they were tested with their fathers or alone, when they were treated with Low AVP and OTA. Compared to when

treated with saline, females long call less when treated with any of the OT treatments and High AVP, suggesting these treatments may have had an anxiolytic effect. The Low and Medium OT doses also significantly explained a lack of long calling for females in the father condition as well, suggesting females are significantly more likely to not long call when treated with these two doses of OT. We had predicted that Low AVP and OTA might have anxiogenic effects, which might increase vocalizations during testing; however, we found the opposite to be true. Indeed, all six of our treatments decreased long calling frequency in females, regardless of whether they were tested with their fathers or alone. While the functionality of titi monkey long calls is still under investigation (Marshall-Ball et al., 2006), one hypothesized purpose is territorial defense (Marshall & Marshall, 1976). While it is more common for adult pairs to long call together (referred to as duetting; Robinson, 1979), we have observed offspring long calling with their parents in captivity both in the present study and during daily scan sampling. If long calling is a form of territorial defense, which could be considered a heightened state of arousal, then our exogenous treatments may have decreased arousal in our females. Further research on the functionality of long calling in non-paired subadults is required to fully understand how manipulations of OT and AVP may be altering this behavior.

Relationship quality also significantly explained variability in long calling behavior in females. If females exhibited a greater increase in vocalizations during IOF testing and in the saline condition of juvenile separation testing, then they tended to long call more in the alone condition. If females spend a greater amount of time in proximity to their fathers during the reunion period following a short-term separation, then females long call more in the alone condition and less in the father condition. These findings suggest that females that have a higher-quality relationship with their fathers, as evidenced by greater distress upon separation and

proximity seeking behaviors, long call more when separated from their fathers, potentially as a way to communicate with the father and solicit his return during the separation period. This finding, in conjunction with our findings regarding the effect of manipulations of the OT and AVP systems on long calling, suggests variability in relationship quality may also impact underlying neurobiology. It is possible that females that form a higher-quality relationship with their fathers have alterations in their OT and AVP systems in such a way that these systems are more likely to be inhibited during temporary separations, resulting in greater long calling behavior, and more likely to be activated when in the presence of their attachment figure, resulting in reduced long calling behavior. A recent study on OT receptor binding in the hippocampal formation found differences in receptor density based on parental status and affiliation between titi monkey pair-mates (Baxter et al., 2020). Therefore, it is possible that the quality of the daughter's relationship with her father may also impact OT receptor density. The present study is one of the first to link relationship quality and receptor manipulations; however, we are unable to assess differences in OT and AVP system functioning in our sample. Future studies should directly investigate how the OT and AVP system is altered by variation in relationship quality in species that form strong, selective attachments.

In addition to vocalization behaviors, we investigated the effects of relationship quality and intranasal manipulations of OT and AVP on locomotion behavior. We studied locomotion because it represents an anxiety-like behavior in non-human primates (NHPs; Barros & Tomaz, 2002; Hennessy et al., 1995). Females locomoted less when tested with their fathers compared to when alone; however, locomotion increased as test number increased, suggesting an effect of time and/or testing experience on locomotion behavior. Females that exhibited a greater increase in line crossing during IOF testing also spent more time locomoting during testing (both in the

father and alone condition). Given that infant locomotion predicted juvenile locomotion, this finding suggests locomotion may measure overall tendencies for females to exhibit "anxiety-like" behaviors during testing that may be consistent within an individual. Indeed, locomotion behavior is used in other nonhuman primate studies as a measure of temperament (Capitanio et al., 2017; Gottlieb et al., 2018) and may reflect stable traits such as general activity level. Interestingly, we found a significant interaction effect between *Infant Carry* and *Condition*. Specifically, females that were carried more by their fathers as infants locomoted less in the father condition but more in the alone condition. These findings mirror our findings for long calling behavior, where females with a higher-quality relationship with their father are potentially more distressed upon separation from their father, as demonstrated by increased locomotor behavior during the separation condition. This heightened distress upon separation is similar to the behaviors exhibited by children with secure attachments during the strange situation paradigm used in humans (for a meta-analysis, see Van Ijzendoorn & Kroonenberg, 1988). Locomotion in titi monkeys, therefore, may be a valuable measure of relationship quality, which appears to remain stable from infancy through adolescence.

Interestingly, our exogenous manipulations of the OT and AVP systems did not significantly affect locomotor behavior. It is unclear why our treatments did not alter locomotor behavior; however, it is possible that locomotion may serve a different function than other behaviors, such as long calling, and therefore other neuropeptide systems may drive variability in locomotor behavior. While the functionality of long calls in titi monkeys is still under investigation, it appears this behavior typically occurs more frequently in tandem with a familiar conspecific than alone, and may also be used to solicit the return of a family member following separation. Therefore, long calls may serve a more social function than behaviors like

locomotion. This particular behavior requires a degree of coordination and synchrony, at least between pair mates (Clink et al., 2019). Research in humans suggests that OT may be particularly important for enhancing social synchrony and coordination of behaviors (Arueti et al., 2013), which may explain why OT manipulations impacted a behavior that required social coordination (e.g., long calling) but not an asocial behavior (e.g., locomotion). It would be helpful to conduct follow up investigations with exogenous manipulations to identify other neuropeptide systems that may better explain variability in asocial behaviors like locomotion.

While OT and AVP manipulations did not significantly alter behavioral correlates of anxiety, we did see interesting effects of treatment and relationship quality on physiological responses to social separations. In particular, females treated with OTA exhibited the largest rise in plasma cortisol compared to the saline condition, regardless of whether they were tested with their father or alone. While blockade of OT receptors disrupted the stress buffering effect of fathers overall, females that spent more time touching the grate during IOF testing exhibited a lower rise in plasma cortisol during juvenile testing. These findings suggest that OTA is able to block the stress-buffering effects the father may have on his daughter, as we had predicted; however, if females have a higher-quality relationship with their fathers, as evidenced by spending more time touching the grate during IOF testing, they may have a reduced physiological response to a social stress paradigm. Therefore, while manipulations of the OT may not significantly impact behavioral expressions of anxiety, blockade of OT receptors is able to enhance physiological separation distress. These findings support the established connections between OT and the HPA-axis (Gobrogge & Wang, 2015). While we did not find support for our prediction that AVP receptor agonism would also exert anxiolytic effects, this was the first study to conduct AVP manipulations in female titi monkeys. It is possible that our treatments were not

sufficient to activate HPA-axis activity. Conversely, our treatments may not have produced any effects if they were large enough to result in AVP binding to both AVP and OT receptors. Our present study is unable to disentangle these two possibilities; therefore, future studies with different dosages of AVP may be useful. It would be particularly important to examine the effects of crosstalk between the OT and AVP receptor systems by studying the dosages at which AVP ligands bind to OT receptors (and vice versa) and how crosstalk between the two systems alters behavior and physiology. This area of research is greatly understudied and warrants further investigation, especially given the potential therapeutic use of OT and AVP receptor ligands (Rae, Devi, Duarte, & Camarini, 2022).

Interestingly, while females that exhibited high grate touching as infants were buffered from the stress of separation, we saw the opposite effect if females spent more time in proximity to their father during IOF testing. This finding suggests these two behaviors (grate touching vs proximity) may be capturing different aspects of females' attachments to their fathers. Touching the grate takes more conscious effort than being in proximity to the father during IOF testing and therefore may be a better indicator of a female's attachment to her father. Indeed, a study examining adult titi monkey behavior during a preference testing paradigm found that females spent more time touching their father's grate than that of a stranger, despite not showing a significant preference for remaining in the father's proximity zone (Oldfield et al., 2019). Alternatively, grate touching may be an indicator of overall anxiety. Therefore, it is important to consider the different functional roles of various expressions of relationship quality.

Eight of our nine potential indicators of relationship quality significantly explained variability in response to a social separation challenge, suggesting it is important to incorporate measures of relationship quality in future studies. Measures of infant attachment were

particularly important for explaining locomotion and plasma CORT concentrations, suggesting early predictors of relationship quality may be particularly useful for understanding variability in behavioral and physiological indicators of anxiety. A study conducted in humans also found that measures of infant attachment predict measures of anxiety later in development (Bar-haim, Dan, & Eshel, 2007). Both juvenile and infant predictors explained variability in vocalizations, particularly long call frequency. It is possible these juvenile indicators of relationship quality account for the greater degree of coordination between fathers and daughters as daughters mature and begin to exhibit more adult-like behaviors. Therefore, it is important to incorporate both infant and current indicators of relationship quality when explaining variability in attachment behaviors at any developmental time point. Our measure of parent affiliation was the only one that did not significantly explain variability in separation distress and stress buffering behavior and physiology. It is possible that the relationship between titi monkey parents does not significantly impact the daughter's attachment to the father. In fact, it is likely that differences in the relationship between the father and daughter may have a stronger impact on the relationship between the parents, as evidenced by a recent study in titi monkeys examining changes in parent affiliation following the birth of an infant (Karaskiewicz et al., 2021). Even though *Parent Affiliation* did not impact separation distress and stress buffering in our subjects, it would be helpful to determine whether or not this predictor explains variability in female proximity maintenance behaviors.

Conclusions. The present study is the first to examine how various indicators of female relationship quality throughout development and manipulations of the OT and AVP systems impact behavioral and physiological correlates of separation distress and stress buffering. Our main objectives were to 1) identify foundational neurobiological and behavioral mechanisms

underlying female stress buffering and separation distress and 2) determine how relationship quality affects bonding mechanisms. Indicators of relationship quality did significantly explain variability in separation distress and stress buffering. In particular, higher-quality relationships with the father both as an infant and a juvenile predicted greater behavioral distress upon separation and enhanced stress buffering physiology. Manipulations of the OT and AVP systems significantly impacted species specific social behavior and physiological indications of separation distress. Specifically, exogenous OT and AVP manipulations reduced long calling behavior while blockade of OT receptors increased CORT production. Interestingly, our treatments did not impact behavioral indicators of separation anxiety, suggesting other neuropeptide systems, like the opioid system, may underlie behavioral correlates of separation distress. Findings from the present studies advance current knowledge of the neurobiological mechanisms foundational to attachment relationships, and help inform how social disruptions caused by events such as the global pandemic may differently impact humans based on the quality of their social bonds.

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TABLES

Table 1. Ethogram for social separation outcome variables.

Behavior	Definition
Separation Distress Vocalization†	Female emits a high-pitched, relatively quiet vocalization (e.g., trill, whistle, or peep)
Long call†	Female emits a loud, low-pitched vocalization (e.g., bellows, pumps) that is typically longer in duration than separation distress vocalizations
Locomotion‡	Female moves at least one body length and lasts until immobile for one second

†Scored as frequency behavior (count)

‡Scored as duration behavior (seconds)

Table 2. Ethogram for relationship quality predictors.

Behavior	Definition
Separation Distress Behaviors	
IOF Locomotion	Percent change in line crossing from the father to the empty box condition of infant open field (IOF) testing
IOF Vocalization	Percent change in vocalizations emitted from the father to the empty box condition of IOF testing
Juvenile Locomotion	Percent change in locomotion behavior from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Juvenile Vocalization	Percent change in total vocalizations emitted from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Proximity Maintenance Behaviors	
Juvenile Proximity	Percentage of time subject spends in proximity, contact, or tail-twining with the father during a 15-minute reunion period immediately following juvenile social separation testing when subjects are treated with saline
IOF Proximity	Percentage of time subject spends in proximity to the father during the father condition of IOF testing
IOF Grate	Percentage of time subject spends touching the grate in the IOF arena during the father condition of IOF testing
Infant Proximity	Percentage of time subject was carried by the father during the first nine months of life as measured during daily scan samples
Parent Affiliation	Percentage of time parents of subjects spent in proximity, contact, or tail-twining with each other during the first 14 months of the subject's life as measured during daily scan samples

Table 3. Pearson correlation matrix of relationship quality predictors. Significant correlations ($p < .05$) indicated in bold. ParAff = *Parent Affiliation;* IOFLine = *IOF Locomotion;* IOFVoc = *IOF Vocalization;* JuvVoc = *Juvenile Vocalization;* JuvLoc = *Juvenile Locomotion;* JuvReun = *Juvenile Proximity;* InfCarry = *Infant Proximity;* IOFProx = *IOF Proximity;* IOFGrate = *IOF Grate*

	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc	JuvReun	InfCarry	IOFProx	IOFGrate
ParAff	1.00								
IOFLine	**-0.48**	1.00							
IOFVoc	**-0.32**	**0.73**	1.00						
JuvVoc	-0.05	**-0.24**	-0.09	1.00					
JuvLoc	**-0.27**	**-0.28**	**-0.25**	**0.42**	1.00				
JuvReun	-0.01	**-0.32**	**-0.25**	-0.07	**0.22**	1.00			
InfCarry	0.04	-0.10	**0.20**	**0.27**	**0.31**	0.00	1.00		
IOFProx	0.05	**0.36**	-0.01	**-0.51**	**-0.43**	**-0.35**	**-0.18**	1.00	
IOFGrate	**-0.30**	0.07	**-0.29**	**-0.40**	**-0.23**	0.00	-0.04	**0.63**	1.00

Table 4. Results from general linear mixed-effects models and zero-inflated mixed-effects models.

4a. Separation Distress Vocalization Frequency

	β	SE	t-value	p
(Intercept)	5.864	0.155	37.912	< .001
ConditionFather	-2.184	0.137	-15.958	< .001
InfCarry	-0.061	0.023	-2.709	0.027
JuvLoc	0.000	0.001	-0.443	0.666
IOFProx	-0.009	0.006	-1.539	0.149
ConditionFather:JuvLoc	0.002	0.000	3.729	< .001
ConditionFather:IOFProx	0.024	0.005	4.386	< .001

4b. Long Call Frequency

Response-part coefficients	β	SE	Z-value	p
(Intercept)	2.024	0.179	11.314	< .001
TreatmentHighAVP	-0.074	0.171	-0.434	0.664
TreatmentHighOT	0.368	0.149	2.476	0.013
TreatmentLowAVP	-0.512	0.171	-3.000	0.003
TreatmentLowOT	0.143	0.143	1.002	0.316
TreatmentMedOT	0.343	0.146	2.345	0.019
TreatmentOTA	-0.415	0.160	-2.590	0.010
ConditionFather	-0.006	0.182	-0.032	0.975
IOFVoc	0.007	0.002	2.927	0.003
JuvVoc	0.001	0.000	5.240	< .001
JuvReun	0.013	0.004	3.481	< .001
TreatmentHighAVP:ConditionFather	-0.592	0.290	-2.040	0.041
TreatmentHighOT:ConditionFather	-0.683	0.282	-2.418	0.016
TreatmentLowAVP:ConditionFather	-0.285	0.274	-1.040	0.298
TreatmentLowOT:ConditionFather	-0.902	0.258	-3.501	< .001
TreatmentMedOT:ConditionFather	-1.019	0.261	-3.899	< .001
TreatmentOTA:ConditionFather	-0.092	0.268	-0.342	0.733
ConditionFather:IOFVoc	-0.003	0.001	-2.718	0.007
ConditionFather:JuvReun	-0.020	0.004	-5.153	< .001
Zero-part coefficients:				
(Intercept)	-1.746	0.913	-1.912	0.056
TreatmentHighAVP	1.727	1.077	1.604	0.109
TreatmentHighOT	1.756	1.075	1.634	0.102
TreatmentLowAVP	0.716	1.140	0.628	0.530
TreatmentLowOT	1.206	1.086	1.110	0.267
TreatmentMedOT	2.118	1.082	1.957	0.050

TreatmentOTA	0.316	1.179	0.268	0.789
ConditionFather	2.054	1.129	1.819	0.069
IOFVoc	-0.002	0.006	-0.320	0.749
TreatmentHighAVP:ConditionFather	-2.052	1.486	-1.381	0.167
TreatmentHighOT:ConditionFather	-1.029	1.493	-0.689	0.491
TreatmentLowAVP:ConditionFather	-2.305	1.620	-1.423	0.155
TreatmentLowOT:ConditionFather	-3.476	1.693	-2.054	0.040
TreatmentMedOT:ConditionFather	-2.984	1.513	-1.973	0.049
TreatmentOTA:ConditionFather	-0.221	1.565	-0.141	0.888
ConditionFather:IOFVoc	-0.019	0.007	-2.594	0.009

4c. Locomotion Duration

	β	SE	t-value	p
(Intercept)	13.961	0.733	19.042	< .001
ConditionFather	-3.307	0.614	-5.390	< .001
TestNo	0.359	0.076	4.701	< .001
InfCarry	0.321	0.071	4.533	< .001
IOFLine	0.007	0.002	3.295	0.009
ConditionFather:InfCarry	-0.405	0.094	-4.288	< .001

4d. Plasma Cortisol

	β	SE	t-value	p
(Intercept)	6.285	0.084	74.673	< .001
TreatmentHighAVP	0.026	0.082	0.315	0.753
TreatmentHighOT	-0.035	0.082	-0.420	0.675
TreatmentLowAVP	0.051	0.082	0.615	0.540
TreatmentLowOT	-0.008	0.082	-0.100	0.920
TreatmentMedOT	-0.013	0.082	-0.158	0.875
TreatmentOTA	0.286	0.082	3.472	< .001
ConditionFather	-0.320	0.044	-7.264	< .001
JuvLoc	0.000	0.000	1.923	0.096
IOFGrate	-0.010	0.004	-2.478	0.042
JuvReun	0.005	0.003	1.824	0.111
IOFProx	0.008	0.003	2.598	0.036

4d. Plasma Cortisol

	β	SE	t-value	p
(Intercept)	6.285	0.084	74.673	< .001
TreatmentHighAVP	0.026	0.082	0.315	0.753
TreatmentHighOT	-0.035	0.082	-0.420	0.675

TreatmentLowAVP	0.051	0.082	0.615	0.540
TreatmentLowOT	-0.008	0.082	-0.100	0.920
TreatmentMedOT	-0.013	0.082	-0.158	0.875
TreatmentOTA	0.286	0.082	3.472	< .001
ConditionFather	-0.320	0.044	-7.264	< .001
JuvLoc	0.000	0.000	1.923	0.096
IOFGrate	-0.010	0.004	-2.478	0.042
JuvReun	0.005	0.003	1.824	0.111
IOFProx	0.008	0.003	2.598	0.036

FIGURES

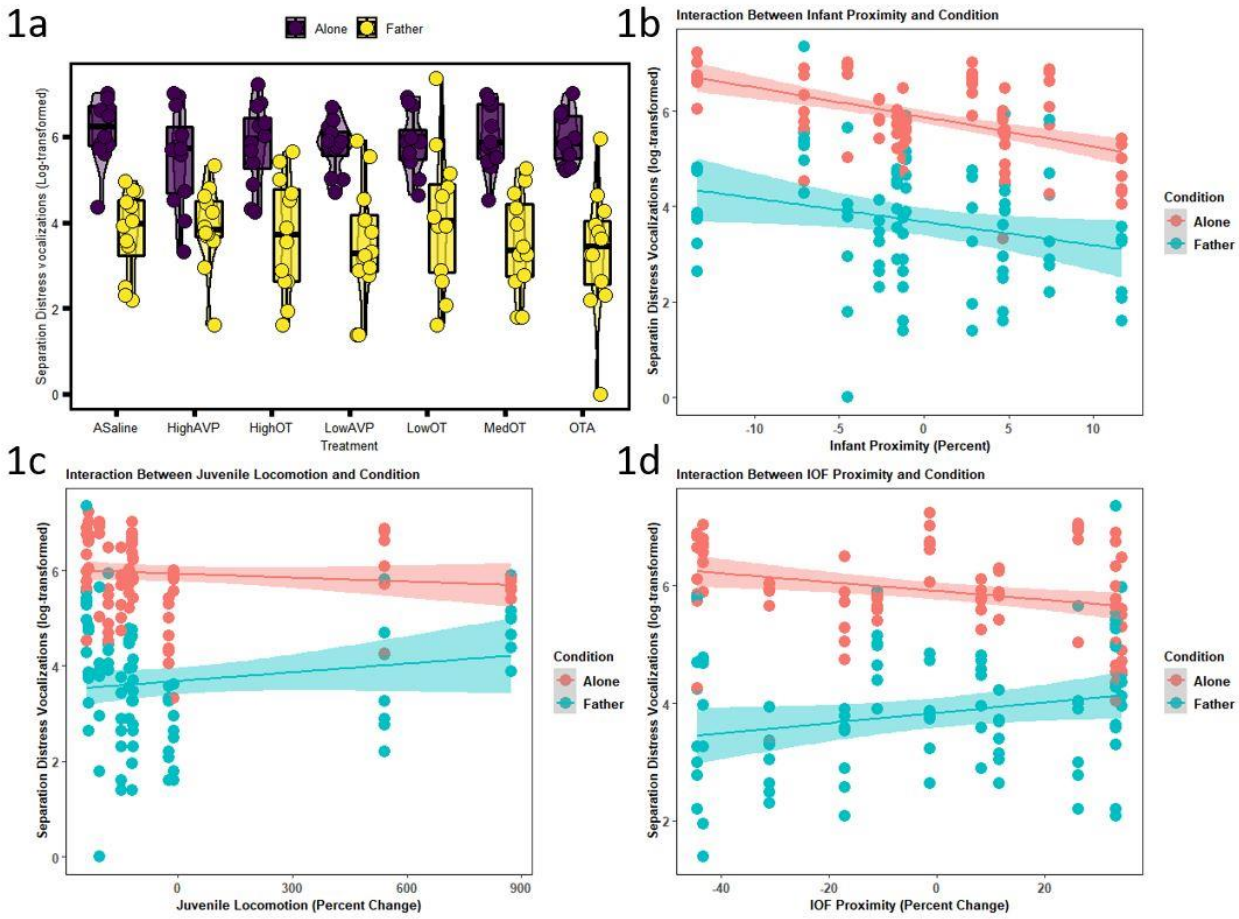


Figure 1. Separation distress vocalization frequency: a) the main effect of *Treatment*, interaction effects between *Condition* and b) *Infant Proximity*, c) *Juvenile Locomotion*, and d) *IOF Proximity*. OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

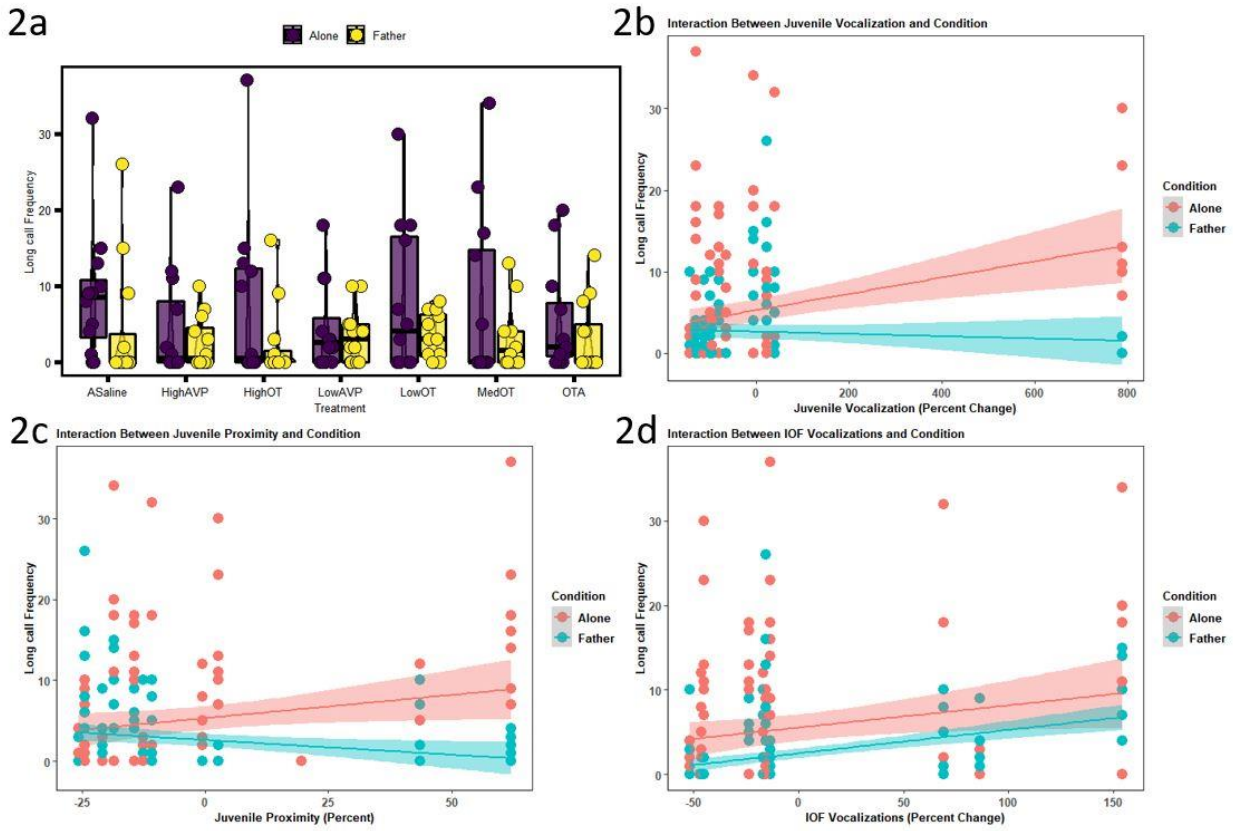


Figure 2. Long call vocalization frequency: a) the main effect of *Treatment*, interaction effects between *Condition* and b) *Juvenile Vocalizations*, c) *Juvenile Proximity*, and d) *IOF Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

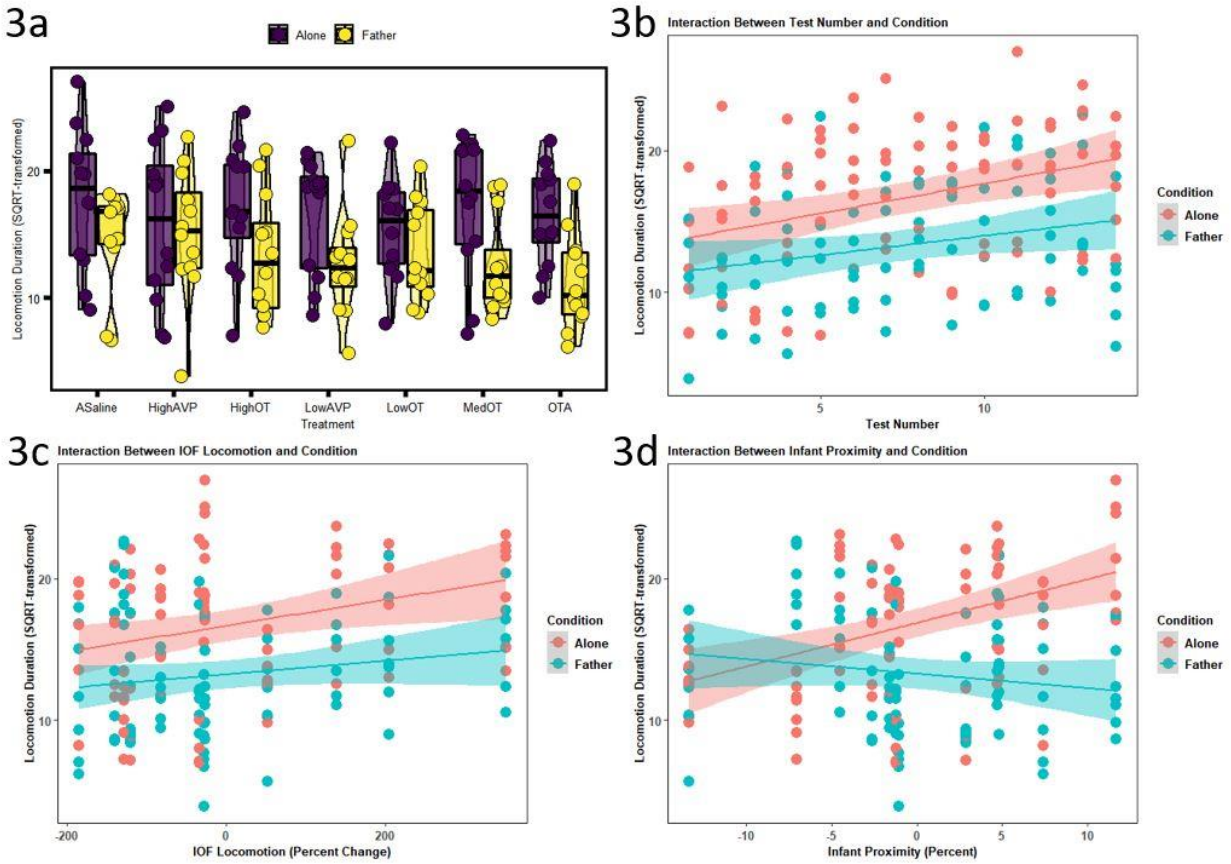


Figure 3. Locomotion duration: a) the main effect of *Treatment*, b) *Test Number*, interaction effects between *Condition* and c) *IOF Locomotion*, and d) *Infant Proximity.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

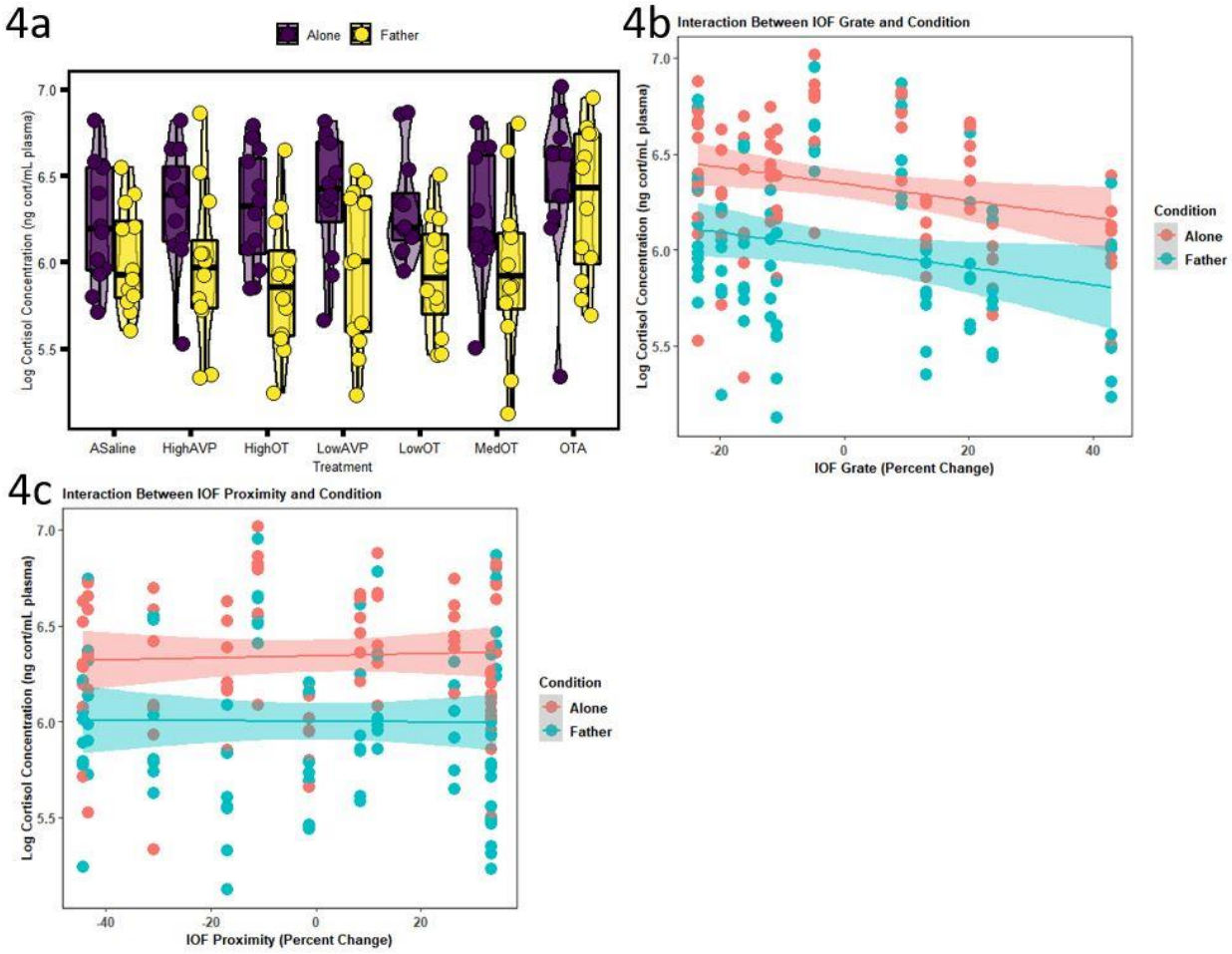


Figure 4. Plasma cortisol concentration: a) the main effect of *Treatment*, interaction effects between *Condition* and b) *IOF Grate*, and c) *IOF Proximity*. OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

SUPPLEMENTARY TABLES

Supplementary Table 1. Log likelihood ratio test results comparing model fit for general linear mixed-effects models and zero-inflated mixed-effects models.

Supplementary 1a. Separation Distress Vocalizations

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
VoconlyMain	7	481.69	503.56	-233.85	467.69			
VoconlySig	9	463.48	491.59	-222.74	445.48	22.2143	2	1.50E-05
VocTwoInt	12	466.13	503.61	-221.06	442.13	3.3523	3	0.3404
VocnoTreat	16	471.18	521.16	-219.59	439.18	2.9463	4	0.5668
VocFull	22	479.18	547.91	-217.59	435.18	4.0004	6	0.6766
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null	3	621.26	630.63	-307.63	615.26			
VoconlySig	9	463.48	491.59	-222.74	445.48	169.78	6	< 2.2e-16

Supplementary 1b. Long Call Vocalizations

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	857.26	880.05	-381.63			
Model2	859.06	878.94	-388.53	13.8	6	0.032
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	859.06	878.94	-388.53			
Model3	854.31	872.73	-389.15	1.25	3	0.7412
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	854.31	872.73	-389.15			
NullModel	994.03	996.45	-492.01	205.72	33	<0.0001

Supplementary 1c. Locomotion Duration

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
LoconlyMain	7	961.87	983.69	-473.93	947.87			
LocOneInt	8	946.03	970.97	-465.01	930.03	17.841	1	2.40E-05
LocnoTreat	9	945.28	973.34	-463.64	927.28	2.743	1	0.09768
LocFull	15	952.3	999.07	-461.15	922.3	4.9817	6	0.54616
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null	3	1005.23	1014.58	-499.61	999.23			
LocOneInt	8	946.03	970.97	-465.01	930.03	69.203	5	1.50E-13

Supplementary 1d. Cortisol

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
CortonlysigMain	12	98.736	136.22	-37.368	74.736			
CortonlyMain	14	93.937	137.67	-32.969	65.937	8.7989	2	0.01228
CortnoVoc	18	100.582	156.81	-32.291	64.582	1.3552	4	0.85195
CortFull	22	97.657	166.38	-26.829	53.657	10.9244	4	0.02743
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null	3	146.583	155.96	-70.292	140.583			
CortonlyMain	14	93.937	137.67	-32.969	65.937	74.646	11	1.58E-11

Supplementary 1a Models:

VoconlyMain: logVoc ~ Condition + InfCarry + JuvLoc + IOFProx + (1 | ID)

VoconlySig: logVoc ~ Condition + InfCarry + JuvLoc + IOFProx + JuvLoc * Condition +

IOFProx * Condition + (1 | ID)

VocTwoInt: logVoc ~ Condition + JuvVoc + InfCarry + JuvLoc + JuvReun + IOFGrate +

IOFProx + JuvLoc * Condition + IOFProx * Condition + (1 |

ID)

VocnoTreat: logVoc ~ Condition + JuvVoc + InfCarry + JuvLoc + JuvReun + IOFGrate +

IOFProx + JuvVoc * Condition + InfCarry * Condition + JuvLoc *

Condition + JuvReun * Condition + IOFGrate * Condition +

IOFProx * Condition + (1 | ID)

VocFull: logVoc ~ Treatment + Condition + JuvVoc + InfCarry + JuvLoc +

JuvReun + IOFGrate + IOFProx + JuvVoc * Condition + InfCarry *

Condition + JuvLoc * Condition + JuvReun * Condition + IOFGrate *

Condition + IOFProx * Condition + (1 | ID)

Null: logVoc ~ 1 + (1 | ID)

Supplementary 1b Models:

Model1<-mixed_model(LongcallF ~ Treatment + Condition + IOFVoc + JuvVoc + JuvReun + IOFGrate +

Treatment*Condition + IOFVoc*Condition + JuvVoc*Condition + JuvReun*Condition +

IOFGrate*Condition,

random = ~ 1|ID,

data = JSS2,

family = zi.poisson(),

zi_fixed = ~ Treatment + Condition + IOFVoc + JuvVoc + JuvReun + IOFGrate +

```
 Treatment*Condition + IOFVoc*Condition + JuvVoc*Condition +
          JuvReun*Condition +
          IOFGrate*Condition,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(LongcallF ~ Treatment + Condition + IOFVoc + JuvVoc + JuvReun +
 Treatment*Condition + IOFVoc*Condition + JuvReun*Condition,
 random = ~ 1|ID,
 data = JSS2,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + Condition + IOFVoc + JuvVoc + JuvReun +
 Treatment*Condition + IOFVoc*Condition + JuvReun*Condition,
 zi_random = ~1|ID,
 iter_EM = 0)
Model3<-mixed_model(LongcallF ~ Treatment + Condition + IOFVoc + JuvVoc + JuvReun +
 Treatment*Condition + IOFVoc*Condition + JuvReun*Condition,
 random = ~ 1|ID,
 data = JSS2,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + Condition + IOFVoc +
 Treatment*Condition + IOFVoc*Condition,
 zi_random = ~1|ID,
 iter_EM = 0)
NullModel<-mixed_model(LongcallF ~ 1,
 random = ~ 1|ID,
 data = JSS2,
 family = zi.poisson(),
 zi_fixed = ~ 1,
```

zi_random = ~1|ID,

iter_EM = 0)

Supplementary 1c Models:

LoconlyMain: sqrtLocoD ~ Condition + TestNo + InfCarry + IOFLine + (1 | ID)

LocOneInt: sqrtLocoD ~ Condition + TestNo + InfCarry + IOFLine + InfCarry * Condition + (1 | ID)

LocnoTreat: sqrtLocoD ~ Condition + TestNo + InfCarry + IOFLine + InfCarry * Condition + IOFLine * Condition + (1 | ID)

LocFull: sqrtLocoD ~ Treatment + Condition + TestNo + InfCarry + IOFLine + InfCarry * Condition + IOFLine * Condition + (1 | ID)

Null: sqrtLocoD ~ 1 + (1 | ID)

Supplementary 1d Models:

CortonlysigMain: logCORT ~ Treatment + Condition + IOFGrate + IOFProx + +(1 | ID)

CortonlyMain: logCORT ~ Treatment + Condition + JuvLoc + IOFGrate + JuvReun + IOFProx + (1 | ID)

CortnoVoc: logCORT ~ Treatment + Condition + JuvLoc + IOFGrate + JuvReun + IOFProx + JuvLoc * Condition + IOFGrate * Condition + JuvReun * Condition + IOFProx * Condition + (1 | ID)

CortFull: logCORT ~ Treatment + Condition + IOFVoc + JuvVoc + JuvLoc + IOFGrate + JuvReun + IOFProx + IOFVoc * Condition + JuvVoc * Condition + JuvLoc * Condition + IOFGrate * Condition + JuvReun * Condition + IOFProx * Condition + (1 | ID)

Null: logCORT ~ 1 + (1 | ID)

CHAPTER 2

The roles of oxytocin, vasopressin, and relationship quality in proximity maintenance in juvenile female titi monkeys

ABSTRACT

Social interactions regulate our behavior and physiology, and strong social bonds can buffer us from stress. Coppery titi monkeys (*Plecturocebus cupreus*) are socially monogamous South American monkeys that display classic attachment behaviors. Infants form selective bonds with their fathers, making them ideal for studying father-daughter bonds. We established a novel method for determining relationship quality in females (n =10). We also investigated how manipulations of oxytocin (OT) and vasopressin (AVP) influenced juvenile attachment. Subjects received acute intranasal treatments of saline, low/medium/high OT, low/high AVP, or OT antagonist (OTA) prior to a parent preference test. Across all parent preference tests, females spent more time in proximity to their parents than the strangers or in the neutral preference zone; however, females exhibited a large degree of individual variation. Manipulations of the OT and AVP systems drove increased proximity maintenance with parents for females that exhibit a higher-quality relationship with their fathers. For example, females that spent more time in proximity to their fathers as infants also spent more time in proximity to their parents when treated with Medium OT ($p = .007$) and High AVP ($p = .002$). Females with a higher-quality father-daughter relationship also spent less time in proximity to strangers when treated with High OT ($p = .003$) and Low OT ($p = .007$), but more time near the strangers when treated with High AVP ($p = .007$), Low AVP ($p = .009$), and OTA ($p = .001$). These findings suggest that when females have a higher quality relationship with their fathers, activation of the OT system reduced willingness to interact with strangers, while blockade of OT receptors and activation of the AVP

system dysregulated species-typical proximity behaviors. While OT and AVP treatments did not impact locomotion, the quality of father-daughter relationships did. Females locomoted more during testing if they spent more time in proximity to their fathers as infants ($p < .001$) and juveniles ($p = .03$) and exhibited more distress upon separation as juveniles ($p < .001$). These findings suggest other neuroendocrine systems may be driving separation distress behaviors. Findings from the present study advance current knowledge of the neurobiological mechanisms foundational to female attachment relationships and help inform how social disruptions may differently impact individuals based on the quality of their social bonds.

INTRODUCTION

Social interactions play a regulatory role in behavioral, psychological, and biological processes (Uchino, 2006). For example, couples in more loving relationships, as measured by the Marital Interaction Scale (Braiker & Kelley, 1979), exhibit a positive correlation between relationship experiences and health habits, which leads to perceptions of better overall health (Markey et al., 2007). Strong social support may buffer individuals from stress-induced behavioral decisions and related physiological changes, such as cardiovascular reactivity (Uchino, 2006). The connection between social well-being and physical health may be one factor contributing to greater longevity of individuals who are happy with their social relationships (Diener & Chan, 2011).

For group-living animals, including humans, a lack of social connections and poor social relationships have been associated with adverse health outcomes and increased risk of mortality (Holt-Lunstad et al., 2010). Poor social relationships in particular have been associated with a 29% increased risk for coronary heart disease, and a 32% increased risk for stroke (Valtorta et al., 2016). Understanding mechanisms underlying social bonds is especially critical now because

the COVID-19 pandemic has detrimentally impacted human social relationships (Evans et al., 2020; Luetke et al., 2020), which may result in adverse health outcomes (Saggioro et al., 2021). Women may be particularly susceptible to psychiatric disorders of social behavior and, compared to men, are more likely to be diagnosed with affective disorders such as major depressive disorder (Weissman & Olfson, 1995). It is possible that social risk factors may contribute to women's increased likelihood of developing affective disorders (Kuehner, 2017). It is therefore important to study how social bonds may affect health outcomes, particularly in women.

A social bond is defined as a close, selective, enduring, interpersonal relationship that is maintained by physiological and behavioral mechanisms (Bales et al., 2021; Lim & Young, 2006). Bowlby (Bowlby, 1977) argued that humans display attachment behaviors (e.g., seeking proximity with preferred individuals, distress upon involuntary separation from attachment figures) throughout their lives, and Ainsworth (Ainsworth et al., 1978) examined social bonds through the lens of attachment theory. Bonded individuals often display physiological and behavioral synchrony, and the degree of this coordination has previously been shown to be predictive of bonding strength in humans (Feldman, 2007, 2012b, 2012a). Various types of social bonds exist across species, including mother-offspring bonds, pair bonds, and friendships.

A monogamous pair bond is an enduring, selective bond between two adults (Bales et al., 2021), exemplified by a preference for close physical proximity to a specific individual (Carp et al., 2016), aggression towards unfamiliar intruders (Winslow et al., 1993), distress upon separation from the pair bonded individual (Mendoza & Mason, 1986a), and the ability of partners to buffer each other from stressors (S. Cohen & Wills, 1985; Heinrichs et al., 2003). Mating also plays a large role in facilitating and maintaining strong affiliative bonds between

adults (Lim & Young, 2006; Numan, 2015), but is not necessary for a pair bond (Bales et al., 2021).

Titi monkeys (*Plecturocebus spp.*) are socially monogamous South American monkeys that live in the Amazon basin (Brazil, Bolivia, Peru, Ecuador, and Colombia). In the wild, titi monkeys live in small family groups and raise their offspring together (Fuentes, 1998). Titi monkeys also display classic attachment behaviors (Fuentes, 1998). For example, titi monkeys maintain proximity with their partners in the lab (Karaskiewicz et al., 2021; Rothwell et al., 2020) and in the wild (Dolotovskaya & Heymann, 2020; Dolotovskaya, Walker, et al., 2020; Kinzey & Wright, 1982), potentially as a means of maintaining pair bonds. Titi monkeys also exhibit distress upon separation from their attachment figures and stress buffering by their attachment figures (Hoffman, 1996; Hoffman et al., 1995; Mendoza & Mason, 1986a; Valeggia, 1996). Because titi monkeys exhibit attachments and family structures similar to humans, they are an excellent model species for research topics with human health applications.

Interestingly, the primary attachment figure for titi monkey infants is the father. The fathers spend more time carrying their infants, and when infants are given a choice between interacting with their mothers or fathers, they will choose their fathers (Mendoza & Mason, 1986b). It is likely that infants form a strong attachment to their fathers because fathers spend more time carrying their infants than mothers (Mendoza & Mason, 1986b). Mothers only tolerate holding infants for feeding, and females have been observed immediately soliciting for the male to take the infant once feeding is done and actively avoiding the infant when not nursing in the wild (Dolotovskaya & Heymann, 2020) and in captivity (Karaskiewicz et al., 2021).

Additional evidence for a selective attachment to the father comes from studies on behavioral and physiological responses to separation from and reunion with the father. Titi

monkey infants will show behavioral signs of distress upon separation from their fathers, but virtually no response when separated from their mothers if the father is present (Hoffman et al., 1995). For example, infants separated from their attachment figures will show increased vocalizations and locomotion (Mendoza & Mason, 1986b), behaviors which serve as measures of anxiety in NHPs (Barros & Tomaz, 2002; Hennessy et al., 1995). Infants also exhibit a significant rise in plasma cortisol (CORT) levels when separated from the father, and only reunion with the father will attenuate that rise in CORT (Mendoza & Mason, 1986b). Anxiety-like behaviors are also attenuated in the presence of the father, but not the mother.

This selective attachment to the father persists as titi monkeys mature. Even at 27 months of age, female titi monkeys exhibit a sustained elevation in CORT throughout a 5-day separation period, and CORT levels return to baseline only upon reunion with parents (Valeggia, 1996). Daughters as old as 3.5 years of age that are reintroduced to natal groups following one month of social separation are able to rejoin their group and similarly show a reduction in hypothalamic-pituitary-adrenal (HPA)-activity following reunion (Hoffman, 1996). Taken together, these findings suggest that the filial bonds between daughters and their father are long-lasting and continue past puberty (previously estimated to occur between 13-15 months [Valeggia, 1996] but more recently estimated to occur closer to 30 months [Conley et al., 2022] for female titi monkeys).

While titi monkeys consistently exhibit a strong, selective attachment to their fathers, there is a large degree of variability in the degree to which they display behavioral correlates of attachment, such as proximity maintenance, both in the lab (Carp et al., 2016; Hoffman, 1996; Mayeaux et al., 2002) and in the wild (Dolotovskaya, Walker, et al., 2020). For example, titi monkeys have demonstrated a preference for maintaining proximity to their parents in tests

conducted at 6, 12, 18, and 24 months of age (Mayeaux et al., 2002), but exhibit a large degree of individual variability within these preference tests. Females between 19 and 48 months of age given the opportunity to interact with unfamiliar conspecifics while in the presence of their parents still prefer to spend the majority of their time in proximity to their parents, but there are some exceptions (Hoffman, 1996). Even offspring as old as 4 years have been observed huddling in affiliative contact with their parents and family members, demonstrating a maintained affiliative relationship between offspring and family even into early adulthood (Mayeaux et al., 2002). However, around this age some titi monkeys have also been observed emigrating from their natal groups in the wild (Bossuyt, 2002; Norconk, 2011; Souza-Alves et al., 2019; Van Belle et al., 2016). After being separated from their family and paired with a male for 1.5 years, females still spend more time interacting with their fathers than a stranger male during a preference test, but this is highly variable across individuals (Carp et al., 2016).

The quality of an offspring's relationship with its father may help explain some of this observed variability in displays of attachment-related behaviors throughout development (e.g., proximity maintenance). This has been demonstrated in humans, where attachment relationships in adolescence have been found to influence adult attachments (Dinero et al., 2008), particularly at the beginning of a new relationship (Dinero et al., 2022). One study has similarly demonstrated an effect of infant attachment on adult relationships in titi monkeys (Savidge & Bales, 2020). In particular, subjects that were more distressed upon separation from their father during an infant social challenge were more affiliative with their partners during the first six months of their first pairing. Subjects that were better buffered from stress by their fathers, as evidenced by decreased anxiety-like behaviors during an infant social challenge, also exhibited lower anxiety-like behaviors in the presence of their partners during a novelty response paradigm

(Savidge & Bales, 2020). It is unknown, however, *how* variation in infant and adolescent relationship quality influences later attachment-related behaviors. It is likely that neurobiological mechanisms important for social bonding and stress buffering underly this variability in attachment behaviors.

Studies of monogamy in prairie voles (*Microtus ochrogaster*) and South American primates, such as owl monkeys (*Aotus spp.*) and titi monkeys (*Plecturocebus spp.*), have demonstrated the importance of oxytocin (OT) and arginine-vasopressin (AVP) for pair bonding (e.g., Bales et al., 2017; Bales, Mason, Catana, Cherry, & Mendoza, 2007; Carter, 1998; Smith, Agmo, Birnie, & French, 2010; Young, Wang, & Insel, 1998). OT and AVP are also involved in human social attachments (Insel & Young, 2001). OT and AVP are both important for approach of novel conspecifics, social recognition, and preferential attachment in males and females (Lim & Young, 2006). Changes in OT and AVP activity during the formation and maintenance of a pair bond have been described extensively in prairie voles and titi monkeys (see Bales et al., 2017).

In addition to OT and AVP, several steroid hormones have also been implicated in the facilitation and maintenance of monogamous pair bonds. One steroid hormone that influences pair bonding is CORT. Female prairie voles require low circulating CORT for the formation of a pair bond, and exhibit high CORT following pair bonding if exposed to a novel male, presumably to prevent her from forming a pair bond with the new male (Devries et al., 1996; K. A. Young et al., 2010). Male prairie voles, on the other hand, require an increase in CORT concentrations to form a pair bond, but will show a decline in CORT when exposed to a novel female regardless of whether or not he was previously pair bonded (K. A. Young et al., 2010).

Understanding the interactions between AVP, OT, and HPA-axis activity may help disentangle some of the sex differences in the neurobiology of attachment.

AVP exerts anxiogenic effects and can be co-secreted with corticotropin-releasing factor (CRF) to potentiate the release of adrenocorticotropic hormone (ACTH; Spencer & Deak, 2016). The period of time spent alone immediately following emigration from a natal group may enhance CORT production as well as AVP production, particularly if the period of separation is prolonged (de Goeij et al., 1991), priming an individual to form a monogamous pair bond. Conversely, OT has been demonstrated to indirectly exert an inhibitory tone on the HPA-axis (Carter et al., 2008) through GABA-ergic signaling (Gobrogge & Wang, 2015). Formation of a pair bond or reunion with a mate following a period of separation may increase OT activity, resulting in an increased inhibition of the HPA-axis. This mechanism seems plausible, given the findings in titi monkeys that separation from a mate was hypothesized to only temporarily dysregulate the HPA-axis due to the rapidness of the return to normal circulating CORT levels upon reunion (Mendoza et al., 2000). Human studies have also demonstrated that intranasal OT in conjunction with social support can reduce salivary CORT levels during a Trier Social Stress Test in men (Heinrichs et al., 2003). OT may therefore be a primary pathway regulating the stress-buffering effects of social support on other physiological systems.

Indeed, administration of OT has been demonstrated to inhibit CORT activity in both male and female prairie voles (Carter, 1998). However, males and females exhibit opposite dose-response curves with regards to the proportion of time they spend in contact with their partners versus a stranger when treated intranasally with OT (Carter et al., 2008). Males spend more time in contact with their partners than strangers when treated with a low (1 mg/kg) or high (4 mg/kg) dose of OT, but spend equal time with strangers and partners when given a medium dose (2

mg/kg). Females, on the other hand, spend more time in contact with their partner when treated with the medium dose, and equal time with the stranger and partner when given the low or high dose of OT (Carter et al., 2008). Further investigation of the interactions between OT, AVP, and CORT may help us better understand the cause of sexual dimorphism in attachment behavior. Females in general have been greatly understudied, and so it is particularly important to investigate the neurobiological mechanism underlying female attachment.

The roles of OT and AVP in titi monkey daughters' responses to separation and reunion with their father are unclear. One way to investigate the role neuropeptides play in attachment-related behaviors is to study the effects of exogenous manipulations of OT and AVP during a social challenge. One challenge with manipulating the OT and AVP system is that these molecules are lipophobic and do not readily cross the blood-brain-barrier. However, the use of intranasal treatments has proven to exert central effects, altering neuronal activity and cognition in humans (for a meta-analysis, see: Bakermans-Kranenburg & Van Ijzendoorn, 2013) and non-human animal models (for a review, see: Freeman & Young, 2016). It is hypothesized that intranasal OT reaches the brain via the trigeminal or olfactory nerve (Veening & Olivier, 2013). A single dose of intranasally administered OT has been shown to be elevated in CSF starting at 15 minutes and lasting from 30-120 minutes (Freeman et al., 2016).

The effects of intranasal OT may be long-lasting due to the feedforward effect of OT where an increase in OT may lead to a further increase in OT (like it does during birth; Bakermans-Kranenburg & Van Ijzendoorn, 2013). Larger doses of OT, however, may have the opposite effect if OT binds to AVP receptors. While OT generally has anxiolytic effects, AVP has a synergistic relationship with CRH and produces anxiogenic effects (Gobrogge & Wang, 2015). OT and AVP have very similar structures and can bind to each other's receptors (Gimpl

& Fahrenholz, 2001). Therefore, if one were to explore the use of OT to reduce anxiety, it would be essential to administer a dosage that was large enough to produce effects but not so large that OT would also activate AVP receptors.

In titi monkeys, as in the prairie voles, chronic treatment with a low dose (0.8 IU/kg OT) facilitates partner preference in adult males but not females (Arias del Razo et al., 2022). Interestingly, chronic intranasal OT increased time spent in proximity with their parents for females but not males (Arias del Razo et al., 2020). Therefore, to better understand the effects of exogenous manipulations of OT on female attachment behaviors, it is important to administer different dosages to investigate a potential dose-response curve.

The effects of acute intranasal AVP treatments have also been investigated in male titi monkeys (Jarcho et al., 2011). This previous study used both a 40 IU/kg and 80 IU/kg dose and found that the higher dose was effective in altering males' behaviors towards their mates. Jarcho and colleagues (Jarcho et al., 2011) used a sequential preference test, presenting males with either their partner, an unfamiliar female, or an empty box. When treated with saline, males spent more time interacting with the stranger females. At the low dose of AVP, males did not show a preference for either female; however, the high dose of AVP resulted in a significant preference for interacting with their partners during the sequential preference test. Given the sex differences observed in OT manipulation studies, it is likely that females may respond differently to manipulations of AVP.

In addition to understanding the effects of exogenous agonists, it is essential to study the effects of antagonists that block receptor activity. There is an oxytocin receptor antagonist (OTA) that is highly selective for OTR in primates and has been used successfully for behavioral pharmacology experiments in marmosets (Cavanaugh et al., 2014). This antagonist has also been

effective in exerting central effects in rhesus macaques when given peripherally through intravenous administration (Boccia et al., 2007). Assays of brain tissue samples 60 minutes post-administration confirmed presence of the compound in the hypothalamus, septum, orbitofrontal cortex, amygdala, and hippocampus. Adult females treated with the OTA exhibited reduced or eliminated interest in infants and sexual behavior (Boccia et al., 2007).

While Cavanaugh and colleagues (Cavanaugh et al., 2014) gave their dose orally and Boccia and colleagues (Boccia et al., 2007) administered their treatment intravenously, Smith and colleagues (A. L. Smith et al., 2017) tracked presence of a similar OTA compound in the plasma and CSF of male rhesus macaques following intranasal administration of the antagonist. The average low dose administered was 205 μg while the average high dose was 7.69 mg. For the high dose, the drug was detectable in the CSF 60- and 120-minutes post-administration. Given the variability in responses to manipulations of the OT system in males and females and the lack of studies on the effects of manipulations of the AVP system in female titi monkeys, it is essential to investigate how exogenous manipulations of these neuropeptide systems impact female attachment-related behaviors. Given the large degree of variability in titi monkeys' responses to social challenges in previous laboratory studies, it is important to investigate how relationship quality affects responses to social challenges. Our main objectives were to understand how relationship quality and manipulations of the OT and AVP systems impact the behavioral and physiological correlates of proximity maintenance.

We had several predictions regarding the impact of relationship quality and neuropeptide manipulations on proximity maintenance (note: the saline treatment condition represents the control condition; therefore, all predictions are in relation to treatment with saline):

1) We predicted a higher-quality relationship with the father, as evidenced by greater distress upon separation and greater proximity maintenance as infants and juveniles, would enhance proximity maintenance. For example, we predicted females with a high-quality relationship with their fathers would spend more time in the preference zone of their parents compared to females with a lower-quality relationship.
2) For OT treatments, we expected a similar dose-response curve as the one observed in prairie voles (Carter et al., 2008), with low and high doses having no or adverse effects with regards to the female's bond with her parents (e.g., no strong preference for maintaining proximity to parents, greater arousal behaviors [e.g., back-arching/tail-lashing] and aggression).
3) We predicted the medium dose would facilitate a preference for the subject's parents, and increase affiliative interactions (e.g., parent-directed lip-smacking).
4) We expected an anxiogenic effect of AVP at the low dose, disrupting preference for the subject's parents and facilitating greater arousal and aggression.
5) If the high dose of AVP results in binding to OTR, then we would expect the high dose of AVP to facilitate a preference for the parents as well as a possible increase in affiliative and arousal behaviors if AVP binding to OTR activated similar behavioral responses.

6) We also predicted OTA would have stress-inducing effects, either abolishing a preference for the parents or facilitating a preference for the stranger. Subjects would also likely display greater behavioral correlates of anxiety.

METHODS

Subjects & Housing

Subjects were juvenile female titi monkeys (ages 18-20 months) and their fathers (N = 10 juvenile females; N = 10 fathers). The age period for the juveniles represents the peri-pubertal stage. Females living in the natal group do not reproduce.

Subjects were housed at the California National Primate Research Center and lived in their natal groups with their parents and any older siblings that were still in the natal group when the subject turned 14 months of age. Families were housed in a 1.2 m x 1.2 m x 2.1 m stainless steel cage or a 1.2 m x 1.2 m x 1.8 m with four horizontal perches, a food bowl, and two water dispensers. They were fed twice daily with Purina New World monkey chow, rice cereal, carrots, apples, and bananas. They were kept on a 12 hours light, 12 hours dark cycle, with lights coming on at 0600 hours and turning off at 1800 hours. The room temperature was maintained at approximately 21°C. For additional information regarding animal husbandry, please see Tardif and colleagues (Tardif et al., 2006) and Mendoza and Mason (Mendoza & Mason, 1986a). This study was approved by the IACUC of the University of California, Davis; and complied with legal requirements of the United States.

Parent Preference Test Data Collection

All subjects experienced seven parent preference tests. At the start of the test, around 0930 hours, subjects were caught in transport cages, manually restrained, and received an intranasal dose of one of the seven treatments (saline, 0.8 IU/kg OT, 8.0 IU/kg OT, 80.0 IU/kg

OT, 40 IU/kg AVP, 80 IU/kg AVP, or 10 mg/kg OTA). Given the variable responses to chronic intranasal OT treatments of 0.8 IU/kg OT (Arias del Razo et al., 2020, 2022), we chose 0.8 IU/kg OT as our lowest dose and chose two higher doses (8.0 IU/kg and 80.0 IU/kg OT) to investigate a potential dose-response curve. These higher doses have been used in rodents without adverse effects (Bales et al., 2013). We selected 40 IU/kg and 80 IU/kg for AVP treatments because those were previously used in male titi monkeys (Jarcho et al., 2011). We chose 10 mg/kg of OTA based on findings from a previous study that used the same OTA intranasally (A. L. Smith et al., 2017) to ensure effectiveness of the treatment while minimizing the risk of adverse side effects. Separation conditions and treatment order were counter-balanced to control for effects of time. Tests were spaced one week apart to ensure drug washout. All treatments were expected to be completely out of the subject's system after 48-hours, and previous studies have tested subjects every other day, so we did not anticipate any carry-over treatment effects one week later (Cavanaugh et al., 2014; Jarcho et al., 2011). We also wanted to keep the developmental period relatively consistent between and within subjects while minimizing stress on the subjects and their families.

Females were then reunited with their families for 30 minutes prior to the start of preference testing. Subjects were released into the center chamber of the testing apparatus (see Figure 1 for testing apparatus) and allowed to freely move about the center arena for the duration of the test. Briefly, tests lasted for approximately 3 hours, and we live-scored five consecutive 30-minute blocks. The subject's parents were on one side of the testing apparatus, and a pair of unfamiliar adults was on the other side (see Figure 1 for testing apparatus). Subjects and stimulus animals remained in their respective area of the testing apparatus for the duration of the test. For a detailed description of preference testing procedures, see Carp and colleagues (Carp et al.,

2016). The side stimulus animals were alternated between tests to avoid development of a side preference.

All tests were video-recorded and live-scored using Behavior Tracker (www.behaviortracker.com) and an established ethogram (Table 1). We quantified the amount of time a subject spent in the preference zone of their parents and the stranger pair. We also scored measures of affiliation (e.g., lip-smacking, long calling), arousal (e.g., back-arching/tail-lashing, chest rubbing), aggression (e.g., exhibiting and receiving aggression), and anxiety (e.g., changing preference zones). For affiliative and aggressive behaviors, we also recorded which stimulus pair the behavior was directed towards. We chose to measure these additional behaviors to get a better understanding of how the treatments may be affecting affiliative and agonistic interactions with stimulus pairs as well as how they affect arousal and stress responses of the subject. At the end of the five observations, we completed five catch and release sessions. We caught the subject, released her into the center arena, and if she stayed in one preference zone for 10 consecutive seconds that was scored as her choice. If she did not stay in either preference zone for 10 consecutive seconds after five minutes, we designate that observation as "no choice". After catch and release, all titi monkeys are returned to their respective home-cage.

Throughout the duration of testing, urine samples were collected three times per week to track subjects' reproductive status. It was not expected that females would be cycling at this age while still in their natal group (Conley et al., 2022; Valeggia, 1996).

Quantification of Relationship Quality

We have access to several measures of infant and juvenile relationship quality in our captive titi monkey colony. For the present study, we quantified several indicators of separation distress and proximity maintenance for our 10 subjects (Table 2). Measures were collected both

as scan samples and as responses to social challenges when females were infants and juveniles. For the data analysis, all measures of relationship quality were centered about the mean value for our 10 subjects. This allowed us to determine how variation in relationship quality influences various outcomes in our testing paradigm. For example, we could examine how females that spend a greater proportion of time in proximity to their fathers as infants differ from females that spend comparatively less time maintaining proximity with their fathers. Below are details regarding data collection for each measure of relationship quality:

IOF Locomotion

All infant titi monkeys at the California National Primate Research Center undergo infant open field (IOF) tests when they are four months of age. This test measures responses to a novel environment and reactions to the presence of family members during a stressful experience. Subjects are placed in the center of a one meter by one meter arena with a six-by-six square floor grid. One wall included a wire mesh grate that allowed auditory, visual, and olfactory access to a stimulus animal that was placed in a transport box on the other side of the grate. During this testing, subjects experienced four five-minute trials, each with a different stimulus on the other side of the grate (their father, their mother, their oldest sibling [if they had any], and an empty box). For a more detailed description of the infant open field methods, see Larke and colleagues (Larke et al., 2017) and Savidge and Bales (Savidge & Bales, 2020). During testing, we counted the number of lines that the infant crossed within each five-minute trial (defined as two or more limbs crossing a line; Larke et al., 2017). For the present study, we counted the number of line crosses for each subject during the empty box condition and the father condition. We then calculated the percent change in line crosses from the father condition to the empty box condition ([{line crossing when alone – line crossing with father / line crossing with father}] *

100). We interpreted a greater increase in line crossing to indicate heightened distress upon separation, which may be an indicator of a higher-quality relationship with the father.

IOF Vocalization

During IOF testing we also score infant vocalizations. During this test we typically only hear infants make trill calls (as defined by Clink et al., 2019), but if they made any other separation distress calls, we combined those with the number of trills scored to create a total vocalization score. We then calculated the percent change in vocalizations from the father condition to the empty box condition. A greater increase in vocalizations was interpreted as an enhanced separation distress response, which is assumed to be an additional indicator of a higher-quality relationship with the father.

Juvenile Locomotion

We quantified measures of juvenile relationship quality using behaviors scored during the saline condition of our social separation testing. We quantified the duration of time females spent locomoting when females were tested alone (separation distress condition) and when they were tested with their father (stress buffering conditions), and calculated the percent change in locomotion from the stress buffering to the separation distress condition. We interpreted a greater increase in locomotion as a heightened separation distress response, which may be indicative of a higher-quality relationship with the father.

Juvenile Vocalization

We also quantified percent change in separation distress vocalization frequency from the stress buffering to the separation distress conditions of the social separation test when females were treated with saline. The values for this measure were much larger than the values for all other relationship quality variables; therefore, we rescaled this measure by dividing by 10 to

make this variable on a more similar scale to our other predictors. A greater increase in vocalizations from the stress buffered to the separation distress condition represented an elevated stress response, which we interpreted as a higher-quality relationship with the father.

Juvenile Proximity

We also quantified measures of juvenile proximity maintenance using video recordings from our social separation testing. Females were reunited with their families in their home-cage immediately following blood sample collection, and their interactions were recorded for 15 minutes. For this measure, we only looked at behaviors during the reunion period following the saline condition of the separation distress condition (when females were left alone in the home-cage for 30 minutes). We calculated the duration of time that females spent in proximity, contact, or tail-twining with their fathers during this 15-minute reunion period (for detailed definitions of these behaviors, see Karaskiewicz et al., 2021). We calculated the percentage of time females were in social proximity with their fathers as the total time in social proximity/contact/tail-twining out of the entire 15-minute reunion period ([duration of time in contact/proximity/tail-twining / 600 seconds] * 100). We interpreted a greater percentage of time maintaining social proximity to the father as a higher-quality relationship with the father.

Juvenile Parent Preference

We quantified additional measures of juvenile proximity maintenance using data from the saline condition of parent preference testing. We calculated the percentage of time females spent in the preference zone of their parents during the entire 3-hour parent preference test by dividing the total time in the parent zone by 150 minutes. We interpreted a greater percentage of time in the parents' preference zone as an indication of a higher-quality relationship with the father.

Juvenile Parent Choice

We also used data from the catch and release sessions when females were treated with saline. We calculated the percentage of times females chose their parents during the five catch and release bouts by dividing the number of times the female chose her parents by five. We assumed females that chose their parents more often had a higher-quality relationship with their father.

IOF Proximity

In addition to quantifying measures of separation distress during IOF testing, we were also able to quantify measures of infant proximity maintenance. We used the same definition of grate proximity that Larke and colleagues (Larke et al., 2017) used to quantify percent time infants spent in close proximity to the grate in the IOF arena. We calculated the percentage of time subjects spent in proximity to the grate during the father condition by dividing the amount of time in proximity to the father by 300 seconds. We assumed greater increase in percentage of time spent in close proximity to the father during testing indicated a higher-quality relationship with the father.

IOF Grate

We also quantified the amount of time the females spent physically touching the grate in the IOF arena. Females had to have at least one hand or foot on the grate to be considered touching the grate (touching the grate with the tail or a different part of the body did not count as grate touching in this test to mirror how grate touching is scored in preference tests [Carp et al., 2016]). We calculated the percentage of time spent touching the grate during the father condition of IOF testing as an additional measure of infant proximity maintenance. We also interpreted a greater percentage of time spent touching the grate as indicating a higher-quality relationship with the father.

Infant Proximity

From the day an infant is born until they are nine months old, we conduct spot checks every two hours, five days per week, to record who is carrying the infant (mother, father, or sibling) or whether the infant is out of contact with any family member and independently moving about the home-cage (Karaskiewicz et al., 2021). The percentage of time spent being carried by a family member out of all checks recorded can give us a measure of the quality of parental care an infant receives. For the present study, we quantified the percentage of time females were carried by their father out of all scan samples collected for that subject. We assume greater percentage of time being carried by the father early in life could be a predictor of a better father-daughter relationship in this species, and possibly result in a higher-quality relationship with the father.

Parent Affiliation

In addition to the infant scan samples, as soon as a titi monkey pair is established, our lab conducts pair-mate scan samples every two hours to record where pair-mates are in relation to each other (Karaskiewicz et al., 2021). The four behaviors recorded on our check sheets are proximity, contact, tail-twining, and nothing. Check sheets are completed five days per week for the entire time that a pair is together. This measure of pair-mate affiliation gives us an indication of the overall level of affiliation that the infant observes in the natal group. In human studies, parents' relationship quality has been found to be a predictor of children's future romantic relationship quality (Gager et al., 2016); therefore, this measure may help us better understand variation in titi monkey attachment. We assume greater percentage of time in affiliative contact is an indicator of higher quality relationships in this species, and may set the foundation for infants to form higher-quality relationships. For the present study, we quantified the percentage

of time parents were in proximity, contact, or tail-twining out of all scan samples collected on the parents during the first 14 months of our subjects' lives.

Data Analysis

All analyses were conducted in R Statistical Software (version 4.0.3, R Core Development Team, 2020). For all outcome measures, we performed a Shapiro Wilk test of normality and transformed non-normally distributed variables as necessary (Royston, 1983). All tests were two-tailed and the significance threshold was set at .05.

We first identified which of the 11 relationship quality variables (*IOF Locomotion, IOF Vocalization, Juvenile Vocalization, Juvenile Locomotion, Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, Infant Proximity, IOF Proximity, IOF Grate, Parent Affiliation;* Table 3) best explained variance in our outcome variables (Table 1). To identify best-fitting relationship quality variables, we ran stepwise regression using the leaps package (Lumley, 2020). This method allowed us to iteratively add and remove variables in the predictive model to identify which subset of variables resulted in the model with the lowest prediction error (Bruce & Bruce, 2017; Gareth et al., 2014). To simplify the stepwise regression models, we first ran separate stepwise regression models for separation distress (*IOF Locomotion, IOF Vocalization, Juvenile Locomotion, Juvenile Vocalization*) and proximity maintenance (*Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, IOF Proximity, IOF Grate, Infant Proximity, Parent Affiliation*) variables. Once we had identified the top separation distress and proximity maintenance variables, we ran a final stepwise regression model that just included those top variables from each category (separation distress and proximity maintenance). The combination of relationship quality variables that was identified as producing a model with the lowest prediction error was then used in our mixed-effects models.

For Zone Ratio, Percent Social, Parent Zone, Stranger Zone, Other Zone, Parent Grate Touch, Stranger Grate Touch, and Zone Change Frequency, we ran general linear mixed-effects models (LMM) using the lmerTest package (Kuznetsova et al., 2017), with animal identity as a random effect to account for repeated measures. Our models were specified as: $y_{ij} = \beta_0 + \beta_1 x^{(1)}_{ij} + \cdots + \beta_m x^{(m)}_{ij} + \eta_{i0} + e_{ij}$. In this model, y_{ij} denotes the *j*th observation taken of individual *i* for outcome variable *y*. Individual covariates included in the model are represented by *x* (covariates 1-m), which have fixed effects common to all individuals in the population (β). This model also includes a random effect of ID that is specific to each individual (η_{i0}). Residual errors are represented by e_{ij}. In our full model, fixed effects included *Treatment*, *Test Number*, *Observation Number*, relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Treatment* and each relationship quality variable. To determine the best-fitting model, we used backwards selection to remove any non-significant fixed effects (Bentler & Mooijaart, 1989). We used a log likelihood ratio test to compare model fit to determine whether removing any non-significant fixed effects resulted in a better fitting model (Vuong, 1989; Supplementary Table 1). We then performed a log likelihood ratio test to compare the fit of our best model to that of the null model where we removed all main effects (Vuong, 1989; West et al., 2012). The resulting final model, determined using a model comparison based on the likelihood of the model to the data, is the only one in which we evaluated the significance of the remaining parameters. Therefore, for each outcome variable, we had one final model where we interpreted significance level. The one final model represented the most likely hypothesized relationship between parameters given the data. Because we only evaluated parameter significance in one model per outcome, post hoc corrections were not necessary (Vuong, 1989; West et al., 2012). For all significant predictors we also calculated Cohen's f^2 as a

measure of effect size (Selya et al., 2012; Snijders & Bosker, 1994). Based on Cohen's (J. Cohen, 1988) guidelines, $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively. We also had strong *a priori* hypotheses regarding how relationship quality variables and treatments would impact our outcome variables. We predicted that higher-quality relationships with the father, as evidenced by greater distress upon separation and proximity maintenance behaviors measured when subjects were infants and juveniles, would result in greater proximity maintenance with the parents. In general, we also predicted agonism of OT receptors would increase affiliative behaviors, antagonism of OT receptors and agonism of AVP receptors would increase arousal and aggression, and very high doses of OT and AVP agonists may result in opposite effects if they bind to each other's' receptors and activate each other's' neural circuitry.

For Partner-directed Lip-smacking, Stranger-directed Lip-smacking, Back-arching/Tail-lashing, and Receive Aggression from Stranger, because there were a large number of observations where females did not display these particular behaviors, we ran zero-inflated models with a Poisson distribution using the GLMMadaptive package (Rizopoulos, 2022). This allowed us to separately model the absence of a behavior and explain variability when females did display that behavior. In our fullest model, we included subject ID as a random effect for both the response and zero-part of the model. We also included the main effects of *Treatment*, *Test Number*, *Observation Number*, relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Treatment* and each relationship quality variable. Similar to our methods for our LMM analyses, we used backwards selection to remove non-significant fixed effects and compared model fit using a log likelihood ratio test. The best-fitting model was compared to a null model, and parameter significance was only

interpreted in that final model. We were unable to calculate Cohen's f^2 for our zero-inflated models.

Behaviors that were scored during testing but occurred too infrequently to model were Chest Rubbing, Long call with Parents, Long call with Strangers, Aggression towards Parents, Aggression towards Strangers, and Receive Aggression from Parents.

RESULTS

Across all parent preference tests, regardless of treatment, females spent more time in proximity to their parents than the strangers or in the neutral preference zone. During an 1800 second observation, females spent an average of 819.43 seconds (SD = 424.21 seconds) in their parents' preference zone, and 290.82 seconds (SD = 296.47 seconds) in the strangers' preference zone. This preference for the parents over the strangers was reflected in a large, positive Zone Ratio score (Mean = 528.61, SD = 567.65). And overall females spent over half of each 30-minute observation in either social preference zone (Mean = 61.90%, SD = 25.73%). Despite this general trend, we observed a large degree of individual variation with regards to how much time females spent in their parents' preference zone. In the present study we sought to explain this variability using measures of infant and juvenile relationship quality.

Zone Ratio

We observed a large degree of variability with regards to where females spent time during preference testing (Figure 2). We wanted to disentangle these individual differences by first examining which zone females spent the most time in. We created a Zone Ratio score by multiplying the time females spent in their parent preference zone by +1, the time in the stranger preference zone by -1, and the time in the neutral zone by 0, and summing these three values per observation. Positive values represented more time in the parent preference zone while negative

values represented more time in the strangers' preference zone. Values closer to zero either indicated a lack of choice between the parents and strangers, with the female spending relatively the same amount of time in each zone, or a preference for the non-social areas of the testing arena.

Residuals for Zone Ratio scores were normally distributed; therefore, no transformations were needed. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, Infant Proximity, IOF Proximity, IOF Grate, Juvenile Vocalizations,* and *Juvenile Locomotion* significantly predicted Zone Ratio scores. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for the main effects of *Test Number* and *Observation Number* (Table 4a).

The R^2 of this model is 0.4511. This model fit significantly better than more parsimonious models tested based on a log likelihood ratio test. Based on our results, across all treatments females tended to spend more time in proximity to their parents than the strangers, but there was some individual variability (Figure 3a). We found a significant, small interaction effect between *Treatment* and *Juvenile Parent Preference* ($f^2 = 0.034$). For females that tended to spend a greater proportion of time in their parents' zone during the saline condition, they also had a more positive zone ratio score (indicating more time in their parents' zone) when treated with High OT (β = -12.835, SE = 5.869, t = -2.187, p = .030; Figure 3b) and Low AVP (β = -13.203, SE = 5.869, t = -2.249, p = .025; Figure 3b). We also found a significant, small interaction effect between *Treatment* and *Juvenile Vocalizations* ($f^2 = 0.017$). For females that vocalize more in the

alone condition compared to the father condition during juvenile social separation testing, they have a more positive zone ratio score when treated with Low AVP (β = -4.183, SE = 2.013, t = 2.078, p = .039; Figure 3c). Finally, we found a significant, small interaction effect between *Treatment* and *Juvenile Locomotion* (f^2 = 0.065). Females that locomote more when separated from their fathers during juvenile testing have a lower zone ratio score which approaches zero when treated with Medium OT (β = -0.837, SE = 0.333, t = -2.516, p = .012; Figure 3d), suggesting they exhibit a lack of choice when given this treatment.

Percent Social

The Zone Ratio score does not distinguish between a female spending a lot of time in "other" and a female spending equal time in parent and stranger zones. To disentangle these two outcomes, we created a Percent Social score, which is the sum of time in the parent preference zone plus the time in the stranger zone divided by the sum of time spent in parent, stranger, and other. We then multiplied that value by 100. Larger values represent a greater percentage of time in a social zone, which would indicate a lack of choice between the parents and strangers if Zone Ratio is close to zero. Smaller values represent a greater proportion of time in the non-social areas of the testing arena.

The residuals were normally distributed for Percent Social scores, so no transformations were needed. Based on stepwise regression analyses, the variables *Juvenile Parent Preference*, *Juvenile Parent Choice, IOF Proximity, IOF Grate, Parent Affiliation,* and *IOF Vocalizations* significantly predicted females' percentage of time in a social zone (either in proximity to the parents or the strangers). We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were

allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for the main effect of *Observation Number* (Table 4b).

The R^2 of this model is 0.6784. This model fit significantly better than more parsimonious models tested based on a log likelihood ratio test. *Treatment* alone did not significantly explain variability in Percent Social scores (Figure 4a). We did find a main effect of test number such that females general spent a greater percentage of time in social zones as test number increased from one to seven ($\beta = 2.34$, SE = 0.747, t = 2.138, p = .002, $f^2 = 0.029$; Figure 4b). We found three statistically significant interaction effects. There was a significant, small interaction effect between *Treatment* and *Juvenile Parent Preference* ($f^2 = 0.057$). For females that spend a greater proportion of time in their parents' preference zone when treated with saline, they spend a greater percentage of time in the social zone when treated with High and Low AVP and High OT, but less time in the social zone when treated with OTA (Table 4b; Figure 4c). There was also a significant, small interaction effect between *Treatment* and *Juvenile Parent Choice* ($f^2 = 0.040$). Females that chose their parents a greater percentage of the time during saline catch and release spent more time in a social zone when treated with OTA but less time when treated with Low OT (Table 4b; Figure 4d). Finally, there was a significant, small interaction effect between *Treatment* and *Parent Affiliation* ($f^2 = 0.018$). For females with parents that spend a greater proportion of time in affiliative contact relative to other parents, they spend more time in social proximity to either their parents or the strangers when treated with Low OT (Table 4b; Figure 4e).

Parent Zone

We were also interested in understanding what factors explain variability in the amount of time females spent within a particular preference zone; therefore, we examined duration of time in each zone separately. The residuals for parent preference zone were normally distributed; therefore, no transformations were needed. Based on stepwise regression analyses, the variables *Juvenile Proximity*, *Juvenile Parent Preference*, *IOF Proximity*, and *Juvenile Vocalizations* significantly predicted parent preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number*, and *Observation Number*. ID was included as a random, repeated measure. *Treatment* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included *Treatment*, the main effects of all four relationship quality variables, as well as the interactions between *Treatment* and all four relationship quality variables (Table 4c).

The R^2 of this model is 0.3862. There was no significant main effect of *Treatment* on its own (Figure 5a); however, when we allowed *Treatment* to interact with all four relationship quality behaviors, all interactions were statistically significant. There was a significant, small interaction effect between *Treatment* and *Juvenile Proximity* ($f^2 = 0.034$): females treated with High AVP spend more time in proximity to their parents if they also spend more time in proximity to their parents during the reunion period following social separation testing (Table 4c; Figure 5b). There was a significant, small interaction effect between *Treatment* and *Juvenile Parent Preference* ($f^2 = 0.067$). For females that typically spend more time in their parents' preference zone during the saline condition compared to other females in the sample, when they are treated with High and Low AVP, OTA, and High OT, they spend significantly more time in proximity to their parents during preference testing (Table 4c; Figure 5c). There was also a

significant, small interaction effect between *Treatment* and *IOF Proximity* (f^2 = 0.038). Females that spend a greater percentage of time in proximity to their fathers during IOF testing also spend a greater amount of time in proximity to their parents when treated with Medium OT and Low AVP (Table 4c; Figure 5d). Finally, there was a significant, small interaction effect between *Treatment* and *Juvenile Vocalizations* (f^2 = 0.060). Females that exhibit a greater increase in vocalizations when separated from their father during social separation testing also spend a greater amount of time in proximity to their parents when treated with High AVP and Medium OT (Table 4c; Figure 5e).

Stranger Zone

The residuals for stranger preference zone duration were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, Infant Proximity, IOF Proximity, IOF Grate, Parent Affiliation, IOF Locomotion,* and *IOF Vocalizations* significantly predicted stranger preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. *Treatment* was allowed to interact with all relationship quality behaviors. The best-fitting model, based on a log likelihood ratio test, included all tested main and interaction effects except for *Observation Number* (Table 4d).

The R^2 of this model is 0.6322. *Treatment* significantly interacted with all relationship quality variables, so we cannot interpret the main effects of *Treatment* on stranger preference zone duration (Figure 6a). In general, females spent more time in proximity to the strangers as test number increased from the first test to the seventh test (β = 5.310, SE = 1.050, t = 3.425, p <

.001, $f^2 < .001$; Figure 6b); however, this effect size is incredibly small so we cannot interpret this finding. We found a significant, large interaction effect between *Treatment* and *Juvenile Proximity* ($f^2 = 1.094$). Females that exhibit a greater amount of time spent in proximity to their fathers during the reunion period after separation testing spent a lower amount of time in proximity to the strangers when treated with High OT and a higher amount when treated with High AVP (Table 4d; Figure 6c). There was also a significant, large effect between *Treatment* and *Juvenile Parent Preference* ($f^2 = 1.585$). For females that generally spend a greater proportion of time in their parents' preference zone when treated with saline, when treated with High or Medium OT and High AVP they spend less time in the strangers' preference zone; however, when treated with Low AVP they spend more time in proximity to the strangers (Table 4d; Figure 6d). We also found a significant, small effect between *Treatment* and *Juvenile Parent Choice* ($f^2 = 0.037$). If a female chose her parents a greater percentage of the time during catch and release when treated with saline, then she spent more time in proximity to the stranger when treated with OTA and High or Low AVP, and less time in the strangers' preference zone when treated with Medium OT (Table 4d; Figure 6e). There was a significant, small effect between *Treatment* and *Infant Proximity* ($f^2 = 0.037$). With regards to females that spent more time being carried by their fathers when they were infants, they spent more time in proximity to the strangers when treated with Low OT and Low AVP but less time when treated with High OT (Table 4d; Figure 6f). We also found a significant, small effect between *Treatment* and *IOF Proximity* ($f^2 = 0.011$). Females that showed a greater increase in time spent in proximity to their fathers during IOF testing spent less time in the strangers' proximity zone when treated with High and Low OT, but more time in proximity to the strangers when treated with High AVP and OTA (Table 4d; Figure 6g). We found a significant, large effect between *Treatment* and *IOF*

Grate (f^2 = 1.418). If females exhibited a greater increase in percent time touching the father's grate compared to the alone condition during IOF testing, then they spent more time in proximity to the strangers when treated with High and Low AVP and OTA, and less time in the strangers' proximity zone when treated with High and Low OT (Table 4d; Figure 6h). We also found a significant, large effect between *Treatment* and *Parent Affiliation* (f^2 = 1.197). If a female's parents spend a greater amount of time in affiliative contact with each other during the first 14 months of the subject's life, then females spend more time in proximity to the strangers when treated with Low OT and Low AVP but less time when treated with High AVP, High OT, and OTA (Table 4d; Figure 6i). There was also a significant, small effect between *Treatment* and *IOF Locomotion* (f^2 = 0.007). For females that exhibit a greater increase in line crossing during IOF testing from the alone to the father condition, females spend less time in the preference zone of the strangers when treated with Low and High AVP but more time in the strangers' preference zone when treated with OTA and High OT (Table 4d; Figure 6j). Finally, we observed a significant, small effect between *Treatment* and *IOF Vocalization* (f^2 = 0.007). If females exhibit a higher increase in vocalizations during IOF testing from the father to the alone condition, they spend less time with the strangers when treated with High and Low AVP (Table 4d; Figure 6k).

Other Zone

Residuals for other preference zone duration were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Preference, Infant Proximity, IOF Proximity, IOF Grate, Parent Affiliation, IOF Locomotion, IOF Vocalizations,* and *Juvenile Vocalizations* significantly predicted other preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and

Observation Number. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all of these main effects except for *Observation Number* as well as the interaction between treatment and all relationship quality variables (Table 4e).

The R^2 of this model is 0.7032. This model fit significantly better than more parsimonious models tested based on a loglikelihood ratio test. We found one main treatment effect ($f^2 = 0.430$). Females spend less time in other when treated with Low AVP ($\beta = -5.74$, SE = 2.55, t = -2.249, p = .025; Figure 7a). We also only found one significant interaction effect between *Treatment* and *Infant Proximity* ($f^2 = 0.273$). Specifically, for females that spent a higher percentage of time being carried by their fathers as infants, if they were treated with Low OT they spent less time in other but if they were treated with OTA they spent more time in other (Table 4e; Figure 7b).

Parent Grate

When females were within the parent or stranger preference zones, they could interact at the grate, which allowed the closest access to the stimulus animal possible. We wanted to know which variables predicted differences in grate-touching behavior in our subjects. The residuals for parent grate touch duration were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, IOF Proximity*, *IOF Grate, Parent Affiliation*, *IOF Locomotion,* and *Juvenile* significantly predicted parent grate touch duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated

measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, was the full model that included all main and interaction effects (Table 4f).

The R^2 of this model is 0.6123, suggesting approximately 61% of the variance is explained by this model. This model fit significantly better than more parsimonious models tested based on a loglikelihood ratio test. Even though our best-fitting model included the main effects of *Test Number* and *Observation Number*, neither statistically significantly predicted parent grate touching duration (Figure 8a). We did find several significant interaction effects between *Treatment* and attachment-related behaviors. We found a significant, small interaction effect between *Treatment* and *Juvenile Proximity* (f^2 = 0.025). Females that spend a greater proportion of time in proximity to their fathers during the reunion period following separation testing spend more time touching their parents' grates when treated with High OT but less time in contact with their parents' grate when treated with OTA (Table 4f; Figure 8b). We also found a significant, small interaction effect between *Treatment* and *Juvenile Parent Preference* (f^2 = 0.085). Females treated with Low AVP spend more time touching their parents' grate if they also spend a greater proportion of time in their parents' preference zone when treated with saline (Table 4f; Figure 8c). There was a significant, small interaction effect between *Treatment* and *Juvenile Parent Choice* (f^2 = 0.076). For females that choose their parents a greater percentage of the time during the saline catch and release testing, females spend more time in contact with their parents' grate when treated with OTA but less if treated with Low OT (Table 4f; Figure 8d). There was also a significant, small interaction effect between *Treatment* and *IOF Proximity* (f^2 = 0.024). Females that exhibit a greater increase in time spent in proximity to their fathers compared to the empty box during IOF testing also exhibit an increase in time spent touching

their parents' grate when treated with Low AVP (Table 4f; Figure 8e). There was a significant, small interaction effect between *Treatment* and *IOF Grate* (f^2 = 0.099). If females show a greater increase in time touching the grate for their father compared to an empty box during IOF testing, then they spend less time touching their parents' grate during preference testing when treated with Low AVP (Table 4f; Figure 8f). We found a significant, small interaction effect between *Treatment* and *IOF Locomotion* (f^2 = 0.094). For females that exhibit a greater increase in line crossing during IOF testing, if they are treated with Low AVP they spend a greater amount of time touching their parents' grate (Table 4f; Figure 8g). Finally, we found a significant, large interaction effect between *Treatment* and *Juvenile Vocalization* (f^2 = 0.943). For females that vocalize more when separated from their fathers during the saline condition of juvenile social separation testing, they spend more time touching their parents' grate if they are treated with High AVP but less time if treated with High OT (Table 4f; Figure 8h).

Stranger Grate

Based on stepwise regression analyses, the variables *Juvenile Parent Preference, IOF Locomotion, IOF Vocalization, Juvenile Vocalization,* and *Juvenile Locomotion* significantly predicted stranger grate touch duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects (Table 4g).

The R^2 of this model is 0.5354. Duration of time spent touching the strangers' grate varied by *Treatment*; however, there were several significant interaction effects so we are not able to interpret the main effects alone (Table 4g; Figure 9a). This model fit significantly better

than more parsimonious models tested based on a loglikelihood ratio test. There were significant main effects of *Test Number* and *Observation Number*. Across tests, as *Test Number* increased from one to seven the amount of time females spent touching the grates of strangers decreased (β = -0.168, SE = 0.053, t = -3.146, p = .002, f^2 = 0.011; Figure 9b). Within tests, as *Observation Number* increased from one to five the amount of time females spent touching the strangers' grate also decreased (β = -0.122, SE = 0.049, t = -2.508, p = .012, f^2 = 0.025; Figure 9c).

We also found several significant interaction effects between *Treatment* and relationship quality variables. For example, we found a significant, small interaction effect between *Treatment* and *Juvenile Parent Preference* (f^2 = 0.013). Females that spend a greater percentage of time in their parents' preference zone in the saline condition spend more time touching the strangers' grate when treated with High OT but less time when treated with High AVP (Table 4g; Figure 9d). We also found a significant, small interaction effect between *Treatment* and *IOF Locomotion* (f^2 = 0.041). For females that exhibit a greater increase in line crossing during IOF testing from the father to the alone condition, they spend a greater amount of time touching the strangers' grate when treated with High OT but less time when treated with Low OT (Table 4g; Figure 9e). There was a significant, small interaction effect between *Treatment* and *IOF Vocalization* (f^2 = 0.014). Females that vocalize more during IOF testing in the alone compared to the father condition during IOF testing spend less time touching the strangers' grate when treated with High AVP and Low OT (Table 4g; Figure 9f). There was also a significant, small interaction effect between *Treatment* and *Juvenile Vocalization* (f^2 = 0.038). Females that vocalize more during the alone compared to the father condition during juvenile social separation saline test spend more time touching the strangers' grate when treated with High OT and High AVP (Table 4g; Figure 9g). Finally, we found a significant, small interaction effect between

Treatment and *Juvenile Locomotion* (f^2 = 0.048). For females that exhibit a greater increase in locomotion during juvenile testing, they spend less time touching the strangers' grate when treated with OTA and High AVP but more time if treated with Medium OT (Table 4g; Figure 9h).

Zone Change Frequency

We used the number of times females changed between the parent, stranger, and other preference zones as a measure of general locomotion during testing. Residuals for Zone Change Frequency were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, Juvenile Parent Choice, IOF Proximity, IOF Grate, Juvenile Vocalizations,* and *Juvenile Locomotion* significantly predicted zone change frequency. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects (Table 4h).

The R^2 of this model is 0.5191. This model fit significantly better than more parsimonious models tested based on a loglikelihood ratio test. *Treatment* had a significant, large effect on Zone Change Frequency (f^2 = 1.041). In general, females changed location less frequently when treated with High AVP and Medium OT (Table 4h; Figure 10a). Females also changed location less frequently as *Observation Number* (β = -0.233, SE = 0.107, t = -2.184, p = .030; f^2 = 0.013; Figure 10b) and *Test Number* (β = -0.473, SE = 0.124, t = -3.829, p < .001; f^2 = 0.044; Figure 10c) increased.

We also found several significant main effects of relationship quality variables. For example, there was a significant, small effect of *Juvenile Parent Preference* (f^2 = 0.090). Females changed preference zones more frequently if they spent a higher proportion of time with their parents during the saline condition (Table 4h; Figure 10d). There was also a significant, large effect of *IOF Grate* (f^2 = 0.374) such that if they exhibited a greater increase in time spent touching their father's grate during IOF testing, they changed zones more frequently (Table 4h; Figure 10e). We found a significant, small effect of *Juvenile Vocalization* (f^2 = 0.099) as well as a significant, large effect of *Juvenile Locomotion* (f^2 = 0.369). Females that exhibited a greater increase in vocalizations during juvenile social separation testing changed zones less frequently during parent preference testing (Table 4h; Figure 10f), whereas females that exhibited a greater increase in locomotion during juvenile testing changed zones more frequently during preference testing (Table 4h; Figure 10g).

The only significant interaction was a small interaction effect between *Treatment* and *IOF Grate* (f^2 = 0.015) which suggested that females that spend a greater amount of time touching their father's grate compared to the empty box grate during IOF testing changed zones less frequently during preference testing when treated with High OT (Table 4h; Figure 10h).

Parent-directed Lip-smacking

We observed a large degree of variability in lip-smacking behavior between females and across tests. We examined when females do and do not lip-smack in the same model to fully capture this variability. Based on stepwise regression analyses, the variables *IOF Proximity* and *IOF Vocalizations* significantly predicted parent-directed lip-smacking. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was

included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all possible main and interaction effects for both the response part and the zero-part of the model (Table 4i).

This full model fit significantly better than any simpler model tested based on a log likelihood ratio test. Even though our model accounts for all main and interaction effects for the zero-part of our model, none of these predictors significantly explained this part of the model. The following results only pertain to when females do lip-smack at their parents. The main effect of *Treatment* alone did not explain variability in parent-directed lip-smacking (Table 4i; Figure 11a). Females lip-smacked significantly less as *Observation Number* increased from one to five ($\beta = -0.634$, SE = 0.105, Z = -6.028, $p < .001$; Figure 11b) and as *Test Number* increased from one to seven ($\beta = -0.605$, SE = 0.116, Z = -5.231, $p < .001$; Figure 11c).

We found two significant interaction effects. We found a significant interaction effect between *IOF Proximity* and *Treatment* such that females that spent more time in proximity to their fathers during IOF testing lip-smacked at their parents less when treated with High and Medium OT and Low AVP, but more when treated with High AVP (Table 4i; Figure 11d). There was also a significant interaction between *Treatment* and *IOF Vocalization.* Females that vocalized more during IOF testing lip-smacked at their parents more when treated with Low and High AVP and Medium OT, but less when treated with High OT (Table 4i; Figure 11e).

Stranger-directed Lip-smacking

Based on stepwise regression analyses, the variables *Juvenile Parent Preference, IOF Proximity,* and *Juvenile Locomotion* significantly predicted stranger-directed lip-smacking. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with

different combinations of these variables with *Treatment*, *Test Number*, and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects as well as random effects for both the response part and zero-part of the model (Table 4j).

This model fit significantly better than all other models tested. For the zero-part of the model, only the interaction between *Treatment* and *IOF Proximity* significantly explained when females did not lip-smack during testing. Specifically, females were more likely to not lip-smack when treated with Low and Medium OT and OTA (Table 4j). With regards to when females do lip-smack at the strangers, the main effect of *Treatment* alone did not significantly explain variability in stranger-directed lip-smacking (Table 4j; Figure 12a). There was significant main effect of *Observation Number* such that females were less likely to lip-smack at the strangers as observation number increased from one to five ($\beta = -0.547$, SE = 0.046, $t = -11.837$, $p < .001$; Figure 12b).

We found three significant interaction effects between *Treatment* and attachment-related behaviors. With regards to the interaction between *Treatment* and *Juvenile Parent Preference*, females that spend a greater percentage of time in proximity to their parents during the saline condition of preference testing lip-smacked more at the strangers when treated with Low OT (Table 4j; Figure 12c). There was also a significant interaction between *Treatment* and *IOF Proximity*. Females that spend more time in proximity to their parents during IOF testing lip-smack at the strangers less when treated with Medium OT (Table 4j; Figure 12d). Finally, we found a significant interaction between *Treatment* and *Juvenile Locomotion.* Females that

locomote more when separated from their fathers during juvenile social separations lip-smacked at the strangers less when treated with OTA (Table 4j; Figure 12e).

Back-arching/Tail-lashing

Back-arching/tail-lashing was another behavior that was highly variable between subjects and across tests. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, IOF Proximity,* and *IOF Vocalizations* significantly predicted back-arching/tail-lashing frequency. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for the main effect of *Test Number* in the response part of the model (the zero-part included all possible main and interaction effects). The zero-part of the model did not include the random effect of ID (Table 4k).

This model fit significantly better than all other models tested. *Treatment* as a main effect alone did not significantly explain back-arching/tail-lashing behavior (Figure 13a). For the zero-part of the model, only *Observation Number* significantly predicted a zero response. Specifically, as observation number increased, females were more likely to not arch, suggesting they tended to only arch in the earlier observations ($\beta = 0.605$, SE = 0.197, $Z = 3.075$, $p = .002$). This supports the relationship between *Observation Number* and arching in the response part of the model, where females are less likely to arch as observation number increases from one to five ($\beta = -0.654$, SE = 0.044, $Z = -14.823$, $p < .001$; Figure 13b).

We found several significant interaction effects between *Treatment* and relationship quality variables for the response part of the model. With regards to the interaction between *Treatment* and *Juvenile Parent Preference,* females that spend a greater proportion of time in proximity to their parents during the saline condition of preference testing also arch more when treated with Low OT and Low AVP (Table 4k, Figure 13c). We also found a significant interaction between *Treatment* and *IOF Proximity.* Females that spend a greater proportion of time in proximity to their father during IOF testing arch/lash less when treated with High OT and Low AVP (Table 4k, Figure 13d). Finally, there was a significant interaction between *Treatment* and *IOF Vocalization.* Females that vocalize more when separated from their father during IOF testing arch/lash more when treated with High and Low OT and Low AVP (Table 4k, Figure 13e).

Receive Aggression from Strangers

Finally, we observed a large amount of variability with regards to whether or not the stranger stimulus animals showed aggression towards our subjects. We included both the presence and absence of subjects receiving aggression from strangers in our models. Based on stepwise regression analyses, the variables *Juvenile Proximity, IOF Locomotion, IOF Vocalization,* and *Juvenile Vocalization* significantly predicted the frequency of receiving aggression from the unfamiliar stimulus pair. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Treatment, Test Number,* and *Observation Number*. ID was included as a random, repeated measure. All relationship quality variables were allowed to interact with *Treatment* in the fullest model. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for *Observation Number* for the response part of the model. For the

zero-part of the model (which explains when females do receive aggression), a model without any predictors but a random effect of ID fit best (Table 4l).

This model fit significantly better than all other models tested. The main effect of *Treatment* alone did not significantly explain variability in aggression received from the stranger pair (Figure 14a). We found a significant main effect of *Test Number* such that females were slightly more likely to receive aggression from strangers as test number increased from one to seven ($\beta = 0.294$, SE = 0.118, Z = 2.481, p = .013; Figure 14b).

We also found several significant interactions between *Treatment* and relationship quality variables. For example, there was a significant interaction between *Treatment* and *Juvenile Proximity.* For females that spend a greater percentage of time in proximity to their fathers following a short separation, treatments with High AVP and Low OT decrease aggression received from strangers whereas Medium OT increases aggression from strangers (Table 4l; Figure 14c). We also found a significant interaction between *Treatment* and *IOF Locomotion.* For females that show a greater percent increase in line crossing during IOF testing in the alone compared to the father condition, they experience increased aggression from strangers when treated with High AVP (Table 4l; Figure 14d). There was also a significant interaction between *Treatment* and *IOF Vocalizations.* Females that exhibit a greater increase in vocalizations during IOF testing when separated from their fathers receive more aggression from strangers when treated with High and Low AVP and Medium OT (Table 4l; Figure 14e). Finally, we found a significant interaction between *Treatment* and *Juvenile Vocalizations* such that females that exhibit a greater increase in distress vocalizations when separated from their father during juvenile social separation testing experience more aggression from strangers when treated with High AVP and less aggression when treated with Low AVP (Table 4l; Figure 14f).

DISCUSSION

We observed a large degree of individual variation with regards to females' behavioral responses to our parent preference testing paradigm. In general, agonism of OT receptors increased time spent in proximity to the parents, whereas agonism of AVP receptors and antagonism of OT receptors increased time spent in proximity to the stranger pair. Interactions between exogenous manipulations of the OT and AVP systems and measures of female relationship quality significantly explained a large percentage of the variability observed in our data (see Table 5 for an overview of interaction effects). Several general patterns emerged the suggested the quality of a daughter's relationship with her father impacted the way she responded to our intranasal treatments.

In particular, interactions between treatments and relationship quality variables helped explain some of the observed variance. For example, females that typically spend a greater amount of time in proximity to their fathers during social challenges as infants and juveniles also spend more time in their parents' preference zone when treated with Medium and High OT, Low and High AVP, and OTA. Females that display greater distress upon separation from their fathers as juveniles also spend more time in proximity to their parents when treated with Medium OT and High AVP. When we examined the amount of time females spent touching the grate of their parents, we found very similar results, where females with higher-quality relationships with their fathers increased time touching their parents' grate when we manipulated the OT and AVP systems. These findings suggest that manipulations of the OT and AVP systems are able to drive increased parent-directed sociality for females that exhibit a higher-quality relationship with their fathers. OT research in humans has similarly identified its role in enhancing affiliative behaviors and preference for in-group members (for a review, see Shamay-Tsoory & Abu-Akel, 2016).

Variation in human relationship quality and pair bonding has been found to be associated with OT receptor expression differences, particularly in women (Walum et al., 2012). It is likely that female titi monkeys exhibit a similar relationship between the quality of their social bonds and OT receptor density. This difference in OT receptor expression may potentially explain variability in females' responsiveness to our exogenous manipulations of the OT system. It is possible that females with higher-quality relationships with their fathers are more sensitive to OT and AVP manipulations, which could explain why these treatments resulted in heightened proximity maintenance with the parents for females with higher-quality relationships. Future studies should investigate differences in OT and AVP receptor density in titi monkeys based on the quality of their relationships with their fathers to test this theory.

While females generally spent less time in the preference zone of the strangers, we continued to observe a large degree of variation with regards to how much time subjects spent in proximity to a pair of unfamiliar adult titi monkeys. In general, females with higher-quality relationships with their fathers, as evidenced by greater time spent in proximity to their fathers during infant and juvenile social challenges, spent less time in proximity to strangers when treated with Low, Medium, and High OT but more time in the strangers' preference zone when treated with Low and High AVP and OTA. These findings suggest that when females have a higher-quality relationship with their fathers, activation of the OT system results in a decrease in willingness to interact with strangers, while blockade of the OT system and activation of the AVP system increases females' time spent in proximity to unfamiliar adult titi monkeys. In this species, offspring typically prefer to spend time in proximity to their attachment figures over strangers (Carp et al., 2016; Hoffman, 1996; Mayeaux et al., 2002). This species-typical behavior appears to be enhanced by activation of the OT system but disrupted by activation of the AVP

system and blockade of the OT system, but specifically for females that exhibit a high-quality relationship with their fathers. These findings further support the idea that the quality of an attachment relationship may be related to differences in underlying neurobiology. The directionality of this relationship is unclear; however, future studies manipulating receptor densities may help identify how a change in receptor availability may alter behavioral expressions of relationship quality.

Interestingly, when we look at the amount of time females spend touching the strangers' grate, we find the opposite pattern, where activation of the OT system increases time touching the stranger's grate, while blockade of OT receptors and activation of the AVP system decreases time spent touching the grate of the unfamiliar pair. A previous preference test study in titi monkey also observed differences in preference zone and grate touching behavior (Carp et al., 2016). It is likely that grate touching reflects a greater drive to interact with a stimulus animal, but may be more strongly influence by the reactions of the stimulus animal than preference zone proximity. Titi monkeys are highly territorial, and the unfamiliar pair of titi monkeys may have viewed the subject as a threat to their bond. As a result, stranger pairs were more likely to display aggression towards subjects if they were in contact with their grate. It is possible that our activation of the OT system caused females to try to interact with the strangers more, as evidenced by increased time touching their grate, but if the strangers were aggressive towards them, they would flee; which may explain the decreased amount of overall time spent in the strangers' zone when treated with OT.

We specifically observed this pattern of increased stranger grate touching in females that have a higher-quality relationship with their fathers, suggesting this higher-quality relationship may make females more willing to try to engage with social others, particularly when their OT

system is activated. Despite this increased willingness to engage with unfamiliar conspecifics, when we looked at the frequency at which females receive aggression from strangers, we do see patterns emerge that help explain why overall time spent in proximity to the strangers was lower for certain treatments. In particular, females with a higher-quality relationship with their father, as evidenced primarily by indicators of infant and juvenile separation distress, received more aggression from strangers when treated with Medium OT. Because females with higher-quality relationships spent more time touching the strangers' grate while also receiving more aggression from strangers, we may interpret this as OT receptor agonism heightening females' drive to interact with social others, despite negative consequences. This may be because OT can function to suppress HPA-axis activity, thereby facilitating the motivation to approach unfamiliar conspecifics (Carter & Altemus, 1997; Heinrichs & Domes, 2008). We found the opposite effect of agonism of the AVP system, where females with higher-quality relationships also received more aggression from strangers when treated with High and Low AVP but spent less time touching the strangers' grate. Given AVP is co-secreted with CRH to potentiate HPA-axis activity (Gobrogge & Wang, 2015), this treatment may have increased females' feelings of stress and therefore decreased their willingness to interact with unfamiliar titi monkeys. Taken together, these findings suggest the OT and AVP systems may be important for interpretation of the salience of social cues and interact with the HPA-axis to differently drive proximity-seeking behaviors with novel conspecifics.

Treatments did not explain variability in the amount of time females spent in the non-social areas of the testing arena or locomotion behavior, suggesting our manipulations of the OT and AVP systems are only impacting social behaviors and not any of the non-social behaviors we measured. Time spent in the non-social areas of the testing arena could indicate heighted

anxiety in titi monkeys, as titi monkeys typically prefer to spend time in close proximity to their attachment figures (Carp et al., 2016; Mendoza & Mason, 1986b; Rothwell et al., 2020). It is possible that other neuropeptide systems are more relevant for mediating certain distress behaviors. For example, a recent review of neurobiological mechanisms related to separation anxiety disorder in humans highlighted the roles of the serotonin, opioid, dopamine, and GABA systems (Schiele et al., 2020). The opioid system in particular is important in mother-infant bonds, and lower levels of endogenous opioids are associated with heightened separation anxiety (Preter & Klein, 2014) while exogenous opioid agonists reduce separation distress responses during social isolation (Herman & Panksepp, 1978). Interestingly, in male titi monkeys, blockade of kappa opioid receptors attenuated separation distress behaviors following a 60-minute separation from their partners (Ragen et al., 2015). Future studies should repeat these preference test paradigms using exogenous opioid manipulations to determine whether female titi monkeys exhibit similar stress buffering effects which may result in heightened willingness to engage with social others.

For the present study, we used the number of times females switched between preference zones as an indicator of general locomotor activity; however, it is difficult to interpret the meaning behind locomotion during the parent preference test. Increased changing of preference zones could indicate an increased willingness to interact with both social pairs (the parents and the strangers). This measure may also indicate greater distress upon separation from the parents during this social challenge. Given that these females tended to spend more time in the social preference zones during testing, we may cautiously interpret these findings as females being more willing to engage with social others if they change zones more frequently. While treatments did not explain variability in female locomotor behavior, several relationship quality

variables were able to explain variability in locomotion during testing. Specifically, females tended to locomote more during testing if they had a higher-quality relationship with their fathers. In particular, there was a large effect of time females spent touching their father's grate during infant testing, where females that spent more time at the grate during IOF testing also changed zones more frequently during the present preference test paradigm. There was a small interaction effect between *Treatment* and *IOF Grate*, where females treated with High OT changed zones less frequently if they scored higher in this indicator of relationship quality. This finding makes sense when taken together with overall time spent in the social zones of the preference test, as High OT treatments increased time in the parents' preference zone and decreased time in the strangers' preference zone when females exhibited higher-quality relationships with their fathers. Because females were spending more time in their parents' proximity zone when given this treatment, they were likely changing zone less frequently, which explains why High OT resulted in lower zone change frequency in these females. Therefore, it is possible that females with higher-quality relationships are more willing to engage with social others, as indicated by increased switching between the two social zones, but activation of the OT system results in heightened preference for interacting specifically with familiar conspecifics.

In addition to examining where females spent time throughout preference testing, we also measured what females were doing during this social challenge. In particular, we measured indicators of affiliation (e.g., lip-smacking), arousal (e.g., back-arching/tail-lashing), and aggression (e.g., aggression towards either stimulus pair). While we had predicted that activation of the AVP system and antagonism of OT receptors would heighten aggression in females, this behavior occurred too infrequently for analyses. It is possible that the suite of behaviors we used

to define titi monkey aggression is more relevant to pair bonded adults. A previous study found that females in newly formed pairs are particularly likely to exhibit aggressive displays during preference testing (Rothwell et al., 2020). Therefore, it would be helpful to conduct these same intranasal manipulations in adult females to better understand interactions between the OT and AVP systems and aggression in titi monkeys.

Lip-smacking has been used as a measure of affiliation in other titi monkey studies (Mercier et al., 2020; Witczak, Ferrer, & Bales, 2018). On average, females lip-smacked more towards the stranger pair of titi monkeys (Mean = 1.41, SD = 4.10) than their parents (Mean = 0.28, SD = 1.22), but this behavior was extremely variable. Interactions between *Treatment* and measures of infant relationship quality significantly explained variability in parent-directed lip-smacking. Specifically, females that maintained greater proximity with the father and exhibited greater distress upon separation during IOF testing lip-smacked at their parents less when treated with High OT, but more when treated with High AVP. Conversely, females that exhibited greater proximity maintenance and distress upon separation from their fathers as juveniles, lip-smacked towards the strangers more when their OT system was activated by Low OT, but less when it was blocked by OTA. Lip-smacking may function to enhance social bonds and reduce feelings of anxiety. If our High AVP and OTA treatments activated the stress response system, then females may have been lip-smacking more towards their parents and less towards the strangers as a means to alleviate feelings of separation distress during testing. If our High and Low OT treatment had anxiolytic effects, then females may not have felt the need to lip-smack towards their parents as frequently, and instead lip-smacked more towards the strangers, particularly when they have a high-quality relationship with their father.

Variation in relationship quality may alter females' responsiveness to exogenous OT manipulations due to changes in the OT system. A recent study on OT receptor binding in the hippocampal formation found differences in receptor density based on parental status and affiliation between titi monkey pair-mates (Baxter et al., 2020). Therefore, it is possible that the quality of the daughter's relationship with her father may also impact OT receptor density. The present study is one of the first to link relationship quality and receptor manipulations; however, we are unable to assess differences in OT and AVP system functioning in our sample. Future studies should directly investigate how the OT and AVP system is altered by variation in relationship quality in species that form strong, selective attachments.

The final behavior we examined was back-arching/tail-lashing (arching/lashing). This behavior was also extremely variable between individuals (Mean = 2.03, SD = 6.92). Interactions between our exogenous treatments and indicators of relationship quality revealed conflicting patterns. For example, we found a positive relation between infant measures of separation distress and arching/lashing when females were treated with OT agonists, but a negative relation between infant measures of proximity maintenance and arching/lashing. Therefore, indicators of separation distress and proximity maintenance may differentially explain arousal behaviors, with greater distress correlating with greater arousal and greater proximity correlating with lower arousal, specifically when the OT system is activated. It is important to continue to examine the effects of these measures of relationship quality separately to best explain variability in responses to social challenges. We also observed opposite interaction effects between treatments and indicators of infant and juvenile proximity maintenance. Greater infant proximity maintenance was negatively related to arching/lashing when females were treated with Low AVP, whereas juvenile proximity maintenance was positively related to arching/lashing for this

same treatment. Therefore, measures of juvenile and infant proximity maintenance may be differently capturing the relationship between variability in relationship quality and underlying neurobiology. It is essential to continue examining these measures of infant and juvenile attachment separately to best explain variability behavioral and physiological mechanisms underlying female social bonds.

All 11 of our indicators of relationship quality significantly explained variability in females' responses to exogenous manipulations of the OT and AVP systems. In general, interactions between treatments and indicators of juvenile attachment seemed to better explain variability in time spent in the parent and stranger preference zones. Interactions between treatment and infant indicators of relationship quality seemed to best explain variability in specific affiliative and arousal behaviors during this testing paradigm. Interestingly, interactions between treatment and *Parent Affiliation* only significantly explained time spent in the stranger preference zone. In general, females of parents that spend a greater percentage of time in affiliative contact spend more time in proximity to strangers when treated with Low OT but less time when treated with High AVP and OTA. This is the opposite of what was generally found for interactions between these three treatments and indicators of infant and juvenile separation distress and proximity maintenance. We may be capturing differences in the effects of *observing* versus *experiencing* high-quality relationships with these different predictors. Females that observe high-quality relationships may be more willing to engage with novel conspecifics if their OT system is activated. This may be due to the anxiolytic properties of OT, where the combination of having observed a high-quality pair bond and activation of OT receptors promotes partner-seeking behaviors in females. While the causes of emigration from the natal group in titi monkeys are still under investigation, it may be important to conduct follow-up

studies to examine how parents' relationships impact pair bond quality in their offspring. Research in humans has demonstrated how parent-child relationship quality impacts adult romantic relationships (for a review, see (River, O'Reilly Treater, Rhoades, & Narayan, 2022); however, the effect of parents' relationship quality with each other on their offspring's later romantic relationships is less well understood and warrants further investigation. Studies that have evaluated this connection have primarily focused on the negative consequences of divorce and conflict between parents (Gager et al., 2016; Rhoades et al., 2012), therefore it would be especially helpful to understand how models of high-quality social relationships impact future social bonds formed by offspring.

Conclusions. The present study is the first to examine how various indicators of female relationship quality throughout development and manipulations of the OT and AVP systems impact behavioral and physiological correlates of proximity maintenance. Our main objectives were to 1) identify foundational neurobiological and behavioral mechanisms underlying female proximity maintenance and 2) determine how relationship quality affects bonding mechanisms. In general, females with a higher-quality relationship with their fathers spent more time in proximity to their parents when treated with High OT and Low AVP, and spent more time with either social preference zone when treated with High OT, Low and High AVP, and OTA. These findings suggest that manipulations of the OT and AVP systems increased females' time spent in proximity to both the parents and the strangers, but especially the parents. Therefore, activation of the OT and AVP systems increase overall sociality in females if they have a higher-quality relationship with their father. In particular, high-quality infant and juvenile father-daughter relationships may set the foundation for forming adult pair bonds if females are more willing to engage with social others. Findings from the present studies advance current knowledge of the

neurobiological mechanisms foundational to attachment relationships, and help inform how social disruptions caused by events such as the global pandemic may differently impact humans based on the quality of their social bonds.

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TABLES

Table 1. Ethogram for parent preference test outcome variables.

Behavior	Definition
Zone Ratio	Ratio indicating which zone (parent, stranger, other) subject spends the most time in. Calculated by multiplying time in parent zone by +1, time in stranger zone by -1, time in other zone by 0, and summing those three values
Percent Social	Percentage of total test time subject is located in either the parent or the stranger zone. Calculated by adding time in parent zone and time in stranger zone, dividing by the total time, and multiplying by 100.
Parent Zone	Total time subject is located in the preference zone next to the parents
Stranger Zone	Total time subject is located in the preference zone next to the stranger pair
Other Zone	Total time subject is located in any location in the test cage other than the parent or stranger preferenc zones
Parent Grate	Total time subject touches the grated window between the test cage and their parents' cage using hands or feet
Stranger Grate	Total time subject touches the grated window between the test cage and the strangers' cage using hands or feet
Zone Change	Total number of times subject changes between parent, stranger, and other zones during testing
Parent-directed Lip-smack	Subject makes rapid lip movement accompanied by smacking sound while facing parents
Stranger-directed Lip-smack	Subject makes rapid lip movement accompanied by smacking sound while facing strangers

Back-arch/Tail-lash	Subject raises dorsal surface of the back in an arched position (sometimes to the point where the front hands come off of the perch, but not limited to this) AND/OR laterally whips the tail (a bout rather than individual lashes).
Chest Rub	Subject displays rubbing of the chest in a variety of forms. This may include rubbing the chest with the hand or rubbing the chest directly onto a perch or wall. The display often includes sniffing of the surface where the chest was rubbed.
Parent Long call	Subject begins long calling within 3 seconds of either parent calling. Test animal must be joining the song of the parent(s).
Stranger Long call	Subject begins long calling within 3 seconds of either stranger calling. Test animal must be joining the song of the stranger(s).
Parent-directed Aggression	Subject displays anything that resembles aggression at the parents' grate. The subject must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.
Stranger-directed Aggression	Subject displays anything that resembles aggression at the strangers' grate. The subject must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.
Receive Aggression from Parent	Parent displays anything that resembles aggression while subject is in the parent zone. The parent must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.

Receive Aggression from Stranger	Stranger displays anything that resembles aggression while subject is in the stranger zone. The stranger must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.

Table 2. Ethogram for relationship quality predictors

Behavior	Definition
Separation Distress Behaviors	
IOF Locomotion	Percent change in line crossing from the father to the empty box condition of infant open field (IOF) testing
IOF Vocalization	Percent change in vocalizations emitted from the father to the empty box condition of IOF testing
Juvenile Locomotion	Percent change in locomotion behavior from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Juvenile Vocalization	Percent change in total vocalizations emitted from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Proximity Maintenance Behaviors	
Juvenile Proximity	Percentage of time subject spends in proximity, contact, or tail-twining with the father during a 15-minute reunion period immediately following juvenile social separation testing when subjects are treated with saline
Juvenile Parent Preference	Percentage of time subject spends in the parent proximity zone of juvenile parent preference tests when subjects are treated with saline
Juvenile Parent Choice	Percentage of times subject chooses parents during the catch and release testing immediately following juvenile parent preference tests when subjects are treated with saline
IOF Proximity	Percentage of time subject spends in proximity to the father during the father condition of IOF testing
IOF Grate	Percentage of time subject spends touching the grate in the IOF arena during the father condition of IOF testing
Infant Proximity	Percentage of time subject was carried by the father during the first nine months of life as measured during daily scan samples

Parent Affiliation	Percentage of time parents of subjects spent in proximity (within ~6 inches of one another), contact (physical body contact), or tail-twining (sitting with tails wrapped together) with each other during the first 14 months of the subject's life as measured during daily scan samples

Table 3. Pearson correlation matrix of relationship quality predictors. Significant correlations ($p < .05$) indicated in bold. IOFLine = *IOF Locomotion;* IOFVoc = *IOF Vocalization;* JuvVoc = *Juvenile Vocalization;* JuvLoc = *Juvenile Locomotion;* JuvReun = *Juvenile Proximity;* PropPar = *Juvenile Preference;* CnRPar = *Juvenile Choice;* InfCarry = *Infant Proximity;* IOFProx = *IOF Proximity;* IOFGrate = *IOF Grate;* ParAff = *Parent Affiliation*

	IOFLine	IOFVoc	JuvVoc	JuvLoc	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff
IOFLine	1.00										
IOFVoc	**0.69**	1.00									
JuvVoc	**0.53**	**0.48**	1.00								
JuvLoc	**-0.20**	**-0.24**	**-0.32**	1.00							
JuvReun	**-0.46**	**-0.36**	**-0.49**	**0.19**	1.00						
PropPar	0.06	**0.33**	**-0.43**	**0.23**	0.05	1.00					
CnRPar	**0.27**	**0.19**	**0.27**	**0.40**	**-0.34**	**0.42**	1.00				
InfCarry	-0.08	**0.23**	**-0.34**	**0.12**	**-0.11**	**0.47**	**-0.15**	1.00			
IOFProx	**0.38**	-0.04	**-0.21**	**-0.20**	**-0.31**	**0.38**	0.06	**0.11**	1.00		
IOFGrate	0.01	**-0.34**	**-0.51**	-0.08	0.07	**0.12**	**-0.27**	**0.14**	**0.56**	1.00	
ParAff	**-0.51**	**-0.32**	**-0.23**	**-0.31**	0.02	-0.01	**-0.18**	0.07	0.03	**-0.33**	1.00

Table 4. Results from general linear mixed-effects models and zero-inflated mixed-effects models

4a. Zone Ratio

	β	SE	t-value	p
(Intercept)	646.681	73.091	8.848	< .001
TreatmentHighAVP	-105.400	88.645	-1.189	0.235
TreatmentHighOT	-230.780	88.645	-2.603	0.010
TreatmentLowAVP	-103.720	88.645	-1.170	0.243
TreatmentLowOT	-99.180	88.645	-1.119	0.264
TreatmentMedOT	-36.585	89.993	-0.407	0.685
TreatmentOTA	-222.900	88.645	-2.515	0.012
PropPar	20.022	4.840	4.137	< .001
InfCarry	-0.146	13.395	-0.011	0.991
IOFProx	-5.133	4.116	-1.247	0.226
IOFGrate	1.299	5.150	0.252	0.803
JuvVoc	-1.692	1.658	-1.021	0.318
JuvLoc	-0.257	0.270	-0.949	0.353
TreatmentHighAVP:PropPar	-3.780	5.869	-0.644	0.520
TreatmentHighOT:PropPar	-12.835	5.869	-2.187	0.030
TreatmentLowAVP:PropPar	-13.203	5.869	-2.249	0.025
TreatmentLowOT:PropPar	-0.485	5.869	-0.083	0.934
TreatmentMedOT:PropPar	5.183	5.953	0.871	0.385
TreatmentOTA:PropPar	-5.506	5.869	-0.938	0.349
TreatmentHighAVP:InfCarry	-20.109	16.238	-1.238	0.217
TreatmentHighOT:InfCarry	9.084	16.238	0.559	0.576
TreatmentLowAVP:InfCarry	-10.273	16.238	-0.633	0.527
TreatmentLowOT:InfCarry	-6.501	16.238	-0.400	0.689
TreatmentMedOT:InfCarry	-14.556	16.361	-0.890	0.374
TreatmentOTA:InfCarry	-31.796	16.238	-1.958	0.051
TreatmentHighAVP:IOFProx	0.477	4.991	0.095	0.924
TreatmentHighOT:IOFProx	1.174	4.991	0.235	0.814
TreatmentLowAVP:IOFProx	2.248	4.991	0.450	0.653
TreatmentLowOT:IOFProx	1.350	4.991	0.270	0.787
TreatmentMedOT:IOFProx	8.118	5.034	1.613	0.108
TreatmentOTA:IOFProx	-4.772	4.991	-0.956	0.340
TreatmentHighAVP:IOFGrate	0.949	6.247	0.152	0.879
TreatmentHighOT:IOFGrate	3.095	6.247	0.495	0.621
TreatmentLowAVP:IOFGrate	10.131	6.247	1.622	0.106
TreatmentLowOT:IOFGrate	-4.505	6.247	-0.721	0.471

TreatmentMedOT:IOFGrate	-6.861	6.368	-1.077	0.282
TreatmentOTA:IOFGrate	7.080	6.247	1.133	0.258
TreatmentHighAVP:JuvVoc	2.276	2.013	1.130	0.259
TreatmentHighOT:JuvVoc	-0.658	2.013	-0.327	0.744
TreatmentLowAVP:JuvVoc	4.183	2.013	2.078	0.039
TreatmentLowOT:JuvVoc	0.124	2.013	0.061	0.951
TreatmentMedOT:JuvVoc	0.740	2.088	0.355	0.723
TreatmentOTA:JuvVoc	-0.505	2.013	-0.251	0.802
TreatmentHighAVP:JuvLoc	-0.090	0.328	-0.273	0.785
TreatmentHighOT:JuvLoc	-0.148	0.328	-0.453	0.651
TreatmentLowAVP:JuvLoc	0.147	0.328	0.448	0.654
TreatmentLowOT:JuvLoc	-0.614	0.328	-1.872	0.062
TreatmentMedOT:JuvLoc	-0.837	0.333	-2.516	0.012
TreatmentOTA:JuvLoc	0.328	0.328	1.002	0.317

4b. Percent Social

	β	SE	t-value	p
(Intercept)	48.530	7.317	6.632	< .001
TreatmentHighAVP	-4.972	3.684	-1.350	0.178
TreatmentHighOT	1.802	3.683	0.489	0.625
TreatmentLowAVP	8.577	3.695	2.321	0.021
TreatmentLowOT	3.391	3.686	0.920	0.358
TreatmentMedOT	2.199	3.987	0.551	0.582
TreatmentOTA	6.718	3.684	1.824	0.069
TestNo	2.344	0.747	3.138	0.002
PropPar	1.008	0.471	2.138	0.093
CnRPar	-0.375	0.572	-0.656	0.544
IOFProx	0.642	0.407	1.579	0.178
IOFGrate	-0.849	0.726	-1.170	0.301
ParAff	-2.335	1.696	-1.377	0.231
IOFVoc	-0.164	0.174	-0.940	0.394
TreatmentHighAVP:PropPar	-0.841	0.254	-3.312	0.001
TreatmentHighOT:PropPar	-0.876	0.252	-3.475	< .001
TreatmentLowAVP:PropPar	-0.610	0.252	-2.418	0.016
TreatmentLowOT:PropPar	0.020	0.252	0.081	0.935
TreatmentMedOT:PropPar	-0.487	0.253	-1.922	0.056
TreatmentOTA:PropPar	-1.283	0.255	-5.023	< .001
TreatmentHighAVP:CnRPar	0.499	0.307	1.628	0.105
TreatmentHighOT:CnRPar	0.264	0.316	0.835	0.404
TreatmentLowAVP:CnRPar	0.451	0.324	1.394	0.164

TreatmentLowOT:CnRPar	-0.702	0.306	-2.297	0.022
TreatmentMedOT:CnRPar	-0.359	0.325	-1.105	0.270
TreatmentOTA:CnRPar	0.870	0.329	2.648	0.009
TreatmentHighAVP:IOFProx	-0.227	0.234	-0.972	0.332
TreatmentHighOT:IOFProx	-0.244	0.229	-1.069	0.286
TreatmentLowAVP:IOFProx	0.107	0.227	0.469	0.639
TreatmentLowOT:IOFProx	-0.423	0.228	-1.858	0.064
TreatmentMedOT:IOFProx	0.154	0.247	0.624	0.533
TreatmentOTA:IOFProx	-0.355	0.233	-1.526	0.128
TreatmentHighAVP:IOFGrate	0.123	0.395	0.310	0.757
TreatmentHighOT:IOFGrate	0.339	0.403	0.840	0.401
TreatmentLowAVP:IOFGrate	0.186	0.391	0.474	0.636
TreatmentLowOT:IOFGrate	0.121	0.393	0.306	0.760
TreatmentMedOT:IOFGrate	-0.594	0.390	-1.522	0.129
TreatmentOTA:IOFGrate	0.681	0.390	1.744	0.082
TreatmentHighAVP:ParAff	0.001	0.949	0.001	1.000
TreatmentHighOT:ParAff	1.052	1.004	1.047	0.296
TreatmentLowAVP:ParAff	0.341	0.949	0.360	0.719
TreatmentLowOT:ParAff	2.213	0.976	2.267	0.024
TreatmentMedOT:ParAff	0.152	0.971	0.157	0.875
TreatmentOTA:ParAff	0.930	0.944	0.985	0.326
TreatmentHighAVP:IOFVoc	-0.098	0.097	-1.008	0.314
TreatmentHighOT:IOFVoc	0.172	0.096	1.779	0.076
TreatmentLowAVP:IOFVoc	0.011	0.097	0.117	0.907
TreatmentLowOT:IOFVoc	0.052	0.097	0.531	0.596
TreatmentMedOT:IOFVoc	0.030	0.097	0.307	0.759
TreatmentOTA:IOFVoc	0.057	0.096	0.596	0.552

4c. Parent Zone

	β	SE	t-value	p
(Intercept)	853.015	54.542	15.640	< .001
TreatmentHighAVP	-87.080	69.111	-1.260	0.209
TreatmentHighOT	-92.920	69.111	-1.345	0.180
TreatmentLowAVP	22.880	69.111	0.331	0.741
TreatmentLowOT	-17.800	69.111	-0.258	0.797
TreatmentMedOT	-5.271	69.778	-0.076	0.940
TreatmentOTA	-46.140	69.111	-0.668	0.505
JuvReun	0.055	2.550	0.021	0.983
PropPar	17.479	3.040	5.750	< .001
IOFProx	-1.130	2.607	-0.433	0.667

JuvVoc	-0.444	1.185	-0.375	0.709
TreatmentHighAVP:JuvReun	8.227	3.229	2.548	0.011
TreatmentHighOT:JuvReun	3.960	3.229	1.227	0.221
TreatmentLowAVP:JuvReun	5.229	3.229	1.620	0.106
TreatmentLowOT:JuvReun	5.144	3.229	1.593	0.112
TreatmentMedOT:JuvReun	5.189	3.237	1.603	0.110
TreatmentOTA:JuvReun	-1.985	3.229	-0.615	0.539
TreatmentHighAVP:PropPar	-9.147	3.850	-2.376	0.018
TreatmentHighOT:PropPar	-11.055	3.850	-2.872	0.004
TreatmentLowAVP:PropPar	-13.797	3.850	-3.584	< .001
TreatmentLowOT:PropPar	-4.774	3.850	-1.240	0.216
TreatmentMedOT:PropPar	-2.307	3.863	-0.597	0.551
TreatmentOTA:PropPar	-14.720	3.850	-3.824	< .001
TreatmentHighAVP:IOFProx	5.028	3.305	1.521	0.129
TreatmentHighOT:IOFProx	2.625	3.305	0.794	0.428
TreatmentLowAVP:IOFProx	8.409	3.305	2.545	0.011
TreatmentLowOT:IOFProx	1.387	3.305	0.420	0.675
TreatmentMedOT:IOFProx	9.065	3.349	2.707	0.007
TreatmentOTA:IOFProx	-0.835	3.305	-0.253	0.801
TreatmentHighAVP:JuvVoc	4.558	1.500	3.038	0.003
TreatmentHighOT:JuvVoc	2.181	1.500	1.454	0.147
TreatmentLowAVP:JuvVoc	2.710	1.500	1.807	0.072
TreatmentLowOT:JuvVoc	1.610	1.500	1.073	0.284
TreatmentMedOT:JuvVoc	4.577	1.504	3.042	0.003
TreatmentOTA:JuvVoc	-1.078	1.500	-0.719	0.473

4d. Stranger Zone

	β	SE	t-value	p
(Intercept)	-8.405	6.257	-1.343	0.998
TreatmentHighAVP	-0.439	1.050	-0.418	0.676
TreatmentHighOT	4.015	1.039	3.866	< .001
TreatmentLowAVP	5.930	1.210	4.901	< .001
TreatmentLowOT	3.581	1.084	3.304	0.001
TreatmentMedOT	-3.210	1.401	-2.291	1.000
TreatmentOTA	5.157	1.050	4.912	< .001
TestNo	5.314	1.552	3.425	< .001
JuvReun	0.020	0.102	0.194	1.000
PropPar	-0.320	0.214	-1.496	1.000
CnRPar	-0.350	0.275	-1.270	1.000
InfCarry	0.180	0.239	0.751	1.000

IOFProx	1.020	0.151	6.765	1.000
IOFGrate	-0.884	0.213	-4.155	1.000
ParAff	-3.380	0.788	-4.292	1.000
IOFLine	-0.120	0.024	-5.062	1.000
IOFVoc	-0.036	0.091	-0.401	1.000
TreatmentHighAVP:JuvReun	-0.577	0.196	-2.946	0.003
TreatmentHighOT:JuvReun	-0.260	0.110	-2.361	0.019
TreatmentLowAVP:JuvReun	0.356	0.228	1.565	0.119
TreatmentLowOT:JuvReun	0.423	0.269	1.575	0.116
TreatmentMedOT:JuvReun	0.676	0.309	2.188	0.969
TreatmentOTA:JuvReun	-0.083	0.067	-1.242	0.215
TreatmentHighAVP:PropPar	1.318	0.515	2.560	0.011
TreatmentHighOT:PropPar	0.839	0.339	2.472	0.014
TreatmentLowAVP:PropPar	-1.536	0.680	-2.259	0.025
TreatmentLowOT:PropPar	-0.647	0.506	-1.281	0.201
TreatmentMedOT:PropPar	-1.415	0.609	-2.322	0.024
TreatmentOTA:PropPar	0.092	0.133	0.692	0.489
TreatmentHighAVP:CnRPar	-0.848	0.378	-2.244	0.026
TreatmentHighOT:CnRPar	0.013	0.217	0.060	0.952
TreatmentLowAVP:CnRPar	2.041	0.723	2.822	0.005
TreatmentLowOT:CnRPar	0.808	0.504	1.605	0.110
TreatmentMedOT:CnRPar	1.617	0.674	2.398	0.018
TreatmentOTA:CnRPar	0.647	0.166	3.907	< .001
TreatmentHighAVP:InfCarry	-0.696	0.367	-1.897	0.059
TreatmentHighOT:InfCarry	-2.007	0.565	-3.553	< .001
TreatmentLowAVP:InfCarry	2.160	0.788	2.742	0.006
TreatmentLowOT:InfCarry	1.892	0.623	3.039	0.003
TreatmentMedOT:InfCarry	1.066	0.512	2.083	0.998
TreatmentOTA:InfCarry	-0.542	0.333	-1.630	0.104
TreatmentHighAVP:IOFProx	-1.706	0.540	-3.158	0.002
TreatmentHighOT:IOFProx	-1.061	0.320	-3.318	0.001
TreatmentLowAVP:IOFProx	0.567	0.308	1.843	0.066
TreatmentLowOT:IOFProx	-0.411	0.182	-2.261	0.025
TreatmentMedOT:IOFProx	0.110	0.194	0.567	1.000
TreatmentOTA:IOFProx	-0.948	0.297	-3.198	0.002
TreatmentHighAVP:IOFGrate	0.436	0.162	2.686	0.008
TreatmentHighOT:IOFGrate	0.889	0.298	2.980	0.003
TreatmentLowAVP:IOFGrate	0.642	0.244	2.634	0.009
TreatmentLowOT:IOFGrate	0.911	0.336	2.710	0.007
TreatmentMedOT:IOFGrate	0.676	0.333	2.028	0.971
TreatmentOTA:IOFGrate	0.564	0.172	3.287	0.001

TreatmentHighAVP:ParAff	2.149	0.777	2.766	0.006
TreatmentHighOT:ParAff	3.662	1.151	3.182	0.002
TreatmentLowAVP:ParAff	1.843	0.627	2.940	0.004
TreatmentLowOT:ParAff	4.004	1.206	3.321	0.001
TreatmentMedOT:ParAff	2.305	1.074	2.145	0.999
TreatmentOTA:ParAff	2.643	0.763	3.464	< .001
TreatmentHighAVP:IOFLine	0.248	0.089	2.785	0.006
TreatmentHighOT:IOFLine	0.113	0.049	2.319	0.021
TreatmentLowAVP:IOFLine	-0.200	0.073	-2.760	0.006
TreatmentLowOT:IOFLine	0.031	0.037	0.840	0.402
TreatmentMedOT:IOFLine	-0.073	0.042	-1.747	1.000
TreatmentOTA:IOFLine	0.104	0.034	3.029	0.003
TreatmentHighAVP:IOFVoc	-0.415	0.150	-2.757	0.006
TreatmentHighOT:IOFVoc	0.010	0.085	0.118	0.906
TreatmentLowAVP:IOFVoc	0.601	0.245	2.457	0.015
TreatmentLowOT:IOFVoc	0.284	0.194	1.460	0.145
TreatmentMedOT:IOFVoc	0.514	0.228	2.254	0.896

4e. Other Zone

	β	SE	t-value	p
(Intercept)	61.510	21.870	2.812	0.999
TreatmentHighAVP	2.502	1.443	1.734	0.084
TreatmentHighOT	-0.468	1.337	-0.350	0.727
TreatmentLowAVP	-5.735	2.550	-2.249	0.025
TreatmentLowOT	-2.592	1.722	-1.505	0.134
TreatmentMedOT	4.492	3.684	1.219	1.000
TreatmentOTA	-2.516	1.443	-1.743	0.082
TestNo	-9.089	5.430	-1.674	0.095
JuvReun	-0.182	0.143	-1.277	1.000
PropPar	0.883	0.748	1.180	1.000
InfCarry	-0.484	0.566	-0.855	1.000
IOFProx	-1.703	1.002	-1.699	1.000
IOFGrate	1.063	0.662	1.606	1.000
ParAff	4.574	2.884	1.586	1.000
IOFLine	0.239	0.155	1.542	1.000
IOFVoc	-0.179	0.161	-1.117	1.000
JuvVoc	0.102	0.137	0.748	1.000
TreatmentHighAVP:JuvReun	0.325	0.259	1.255	0.210
TreatmentHighOT:JuvReun	0.249	0.185	1.343	0.180
TreatmentLowAVP:JuvReun	0.286	0.146	1.952	0.052

TreatmentLowOT:JuvReun	-0.577	0.311	-1.858	0.064
TreatmentMedOT:JuvReun	-0.435	0.256	-1.699	1.000
TreatmentOTA:JuvReun	0.673	0.354	1.899	0.059
TreatmentHighAVP:PropPar	0.041	0.137	0.300	0.764
TreatmentHighOT:PropPar	-1.058	0.820	-1.291	0.198
TreatmentLowAVP:PropPar	-1.747	1.203	-1.452	0.148
TreatmentLowOT:PropPar	-0.792	0.723	-1.096	0.274
TreatmentMedOT:PropPar	-1.577	1.217	-1.296	0.196
TreatmentOTA:PropPar	-1.301	0.952	-1.366	0.173
TreatmentHighAVP:InfCarry	0.229	0.315	0.726	0.468
TreatmentHighOT:InfCarry	2.689	1.772	1.517	0.130
TreatmentLowAVP:InfCarry	-1.530	1.079	-1.419	0.157
TreatmentLowOT:InfCarry	-1.906	0.963	-1.981	0.049
TreatmentMedOT:InfCarry	0.088	0.362	0.242	1.000
TreatmentOTA:InfCarry	2.354	1.152	2.044	0.042
TreatmentHighAVP:IOFProx	1.501	0.986	1.523	0.129
TreatmentHighOT:IOFProx	1.504	1.003	1.499	0.135
TreatmentLowAVP:IOFProx	1.344	0.973	1.382	0.168
TreatmentLowOT:IOFProx	1.377	1.011	1.362	0.174
TreatmentMedOT:IOFProx	1.718	1.420	1.210	0.999
TreatmentOTA:IOFProx	2.184	1.365	1.600	0.111
TreatmentHighAVP:IOFGrate	-0.609	0.361	-1.689	0.092
TreatmentHighOT:IOFGrate	-1.495	0.895	-1.671	0.096
TreatmentLowAVP:IOFGrate	-0.869	0.633	-1.372	0.171
TreatmentLowOT:IOFGrate	-1.601	1.003	-1.596	0.112
TreatmentMedOT:IOFGrate	-1.307	0.980	-1.334	0.992
TreatmentOTA:IOFGrate	-0.565	0.349	-1.621	0.106
TreatmentHighAVP:ParAff	-3.055	2.134	-1.432	0.153
TreatmentHighOT:ParAff	-5.835	3.818	-1.528	0.128
TreatmentLowAVP:ParAff	-3.363	2.535	-1.326	0.186
TreatmentLowOT:ParAff	-7.162	4.453	-1.609	0.109
TreatmentMedOT:ParAff	-5.770	4.184	-1.379	0.999
TreatmentOTA:ParAff	-3.392	2.415	-1.405	0.161
TreatmentHighAVP:IOFLine	-0.195	0.147	-1.330	0.185
TreatmentHighOT:IOFLine	-0.179	0.141	-1.268	0.206
TreatmentLowAVP:IOFLine	-0.167	0.128	-1.311	0.191
TreatmentLowOT:IOFLine	-0.208	0.182	-1.143	0.254
TreatmentMedOT:IOFLine	-0.267	0.240	-1.117	0.999
TreatmentOTA:IOFLine	-0.284	0.194	-1.463	0.145
TreatmentHighAVP:IOFVoc	0.011	0.061	0.186	0.852
TreatmentHighOT:IOFVoc	-0.022	0.060	-0.363	0.717

TreatmentLowAVP:IOFVoc	0.420	0.270	1.559	0.120
TreatmentLowOT:IOFVoc	0.033	0.110	0.297	0.767
TreatmentMedOT:IOFVoc	0.257	0.254	1.012	0.998
TreatmentOTA:IOFVoc	0.374	0.234	1.596	0.112
TreatmentHighAVP:JuvVoc	0.210	0.223	0.943	0.347
TreatmentHighOT:JuvVoc	-0.034	0.055	-0.612	0.541
TreatmentLowAVP:JuvVoc	-0.564	0.364	-1.549	0.123
TreatmentLowOT:JuvVoc	-0.388	0.230	-1.692	0.092
TreatmentMedOT:JuvVoc	-0.597	0.335	-1.784	0.082

4f. Parent Grate Touch

	β	SE	t-value	p
(Intercept)	23.540	6.007	3.918	1.000
TreatmentHighAVP	-0.717	0.997	-0.719	0.473
TreatmentHighOT	-0.787	0.986	-0.799	0.425
TreatmentLowAVP	-0.104	1.149	-0.091	0.928
TreatmentLowOT	0.350	1.029	0.340	0.734
TreatmentMedOT	-0.536	1.366	-0.392	1.000
TreatmentOTA	1.835	0.997	1.841	0.067
ObsNo	0.345	0.186	1.853	0.065
TestNo	-2.576	1.473	-1.749	0.082
JuvReun	0.126	0.062	2.044	1.000
PropPar	0.144	0.141	1.019	1.000
CnRPar	0.260	0.118	2.202	1.000
InfCarry	0.227	0.244	0.932	1.000
IOFProx	-0.211	0.227	-0.929	1.000
IOFGrate	0.277	0.215	1.284	1.000
ParAff	0.704	0.820	0.858	1.000
IOFLine	0.053	0.031	1.715	1.000
JuvVoc	-0.028	0.041	-0.670	1.000
TreatmentHighAVP:JuvReun	0.163	0.088	1.848	0.066
TreatmentHighOT:JuvReun	0.148	0.067	2.200	0.029
TreatmentLowAVP:JuvReun	0.023	0.062	0.371	0.711
TreatmentLowOT:JuvReun	-0.101	0.122	-0.826	0.410
TreatmentMedOT:JuvReun	-0.119	0.137	-0.868	1.000
TreatmentOTA:JuvReun	-0.198	0.064	-3.105	0.002
TreatmentHighAVP:PropPar	-0.142	0.103	-1.377	0.170
TreatmentHighOT:PropPar	-0.341	0.234	-1.457	0.146
TreatmentLowAVP:PropPar	-0.343	0.145	-2.372	0.018
TreatmentLowOT:PropPar	-0.155	0.157	-0.988	0.324

TreatmentMedOT:PropPar	-0.183	0.161	-1.136	1.000
TreatmentOTA:PropPar	0.006	0.126	0.044	0.965
TreatmentHighAVP:CnRPar	0.153	0.097	1.571	0.117
TreatmentHighOT:CnRPar	-0.082	0.094	-0.874	0.383
TreatmentLowAVP:CnRPar	-0.187	0.182	-1.024	0.307
TreatmentLowOT:CnRPar	-0.258	0.107	-2.404	0.017
TreatmentMedOT:CnRPar	-0.370	0.174	-2.124	1.000
TreatmentOTA:CnRPar	-0.325	0.157	-2.065	0.040
TreatmentHighAVP:InfCarry	-0.033	0.239	-0.136	0.892
TreatmentHighOT:InfCarry	0.697	0.520	1.341	0.181
TreatmentLowAVP:InfCarry	-0.108	0.451	-0.239	0.811
TreatmentLowOT:InfCarry	0.016	0.371	0.044	0.965
TreatmentMedOT:InfCarry	0.032	0.272	0.118	1.000
TreatmentOTA:InfCarry	-0.509	0.316	-1.611	0.108
TreatmentHighAVP:IOFProx	0.345	0.295	1.171	0.243
TreatmentHighOT:IOFProx	0.370	0.276	1.341	0.181
TreatmentLowAVP:IOFProx	0.350	0.166	2.109	0.036
TreatmentLowOT:IOFProx	0.273	0.246	1.107	0.269
TreatmentMedOT:IOFProx	0.374	0.284	1.316	1.000
TreatmentOTA:IOFProx	0.084	0.282	0.297	0.767
TreatmentHighAVP:IOFGrate	-0.084	0.156	-0.540	0.590
TreatmentHighOT:IOFGrate	-0.483	0.282	-1.710	0.088
TreatmentLowAVP:IOFGrate	-0.450	0.221	-2.038	0.043
TreatmentLowOT:IOFGrate	-0.391	0.311	-1.259	0.209
TreatmentMedOT:IOFGrate	-0.411	0.307	-1.339	0.999
TreatmentOTA:IOFGrate	-0.076	0.163	-0.465	0.643
TreatmentHighAVP:ParAff	-0.546	0.673	-0.812	0.418
TreatmentHighOT:ParAff	-1.226	1.097	-1.118	0.265
TreatmentLowAVP:ParAff	-1.186	0.727	-1.631	0.104
TreatmentLowOT:ParAff	-1.138	1.252	-0.909	0.364
TreatmentMedOT:ParAff	-1.387	1.157	-1.199	1.000
TreatmentOTA:ParAff	-0.686	0.724	-0.947	0.345
TreatmentHighAVP:IOFLine	-0.079	0.042	-1.853	0.065
TreatmentHighOT:IOFLine	-0.053	0.037	-1.438	0.152
TreatmentLowAVP:IOFLine	-0.042	0.016	-2.572	0.011
TreatmentLowOT:IOFLine	-0.058	0.041	-1.393	0.165
TreatmentMedOT:IOFLine	-0.062	0.044	-1.421	1.000
TreatmentOTA:IOFLine	-0.047	0.033	-1.426	0.155
TreatmentHighAVP:JuvVoc	0.222	0.054	4.099	< .001
TreatmentHighOT:JuvVoc	0.076	0.031	2.475	0.014
TreatmentLowAVP:JuvVoc	-0.035	0.088	-0.399	0.691

TreatmentLowOT:JuvVoc	0.003	0.070	0.050	0.960
TreatmentMedOT:JuvVoc	0.004	0.082	0.053	0.999

4g. Stranger Grate Touch

	β	SE	t-value	p
(Intercept)	3.268	0.401	8.145	< .001
TreatmentHighAVP	0.173	0.257	0.671	0.503
TreatmentHighOT	0.666	0.257	2.589	0.010
TreatmentLowAVP	0.831	0.258	3.218	0.001
TreatmentLowOT	0.745	0.257	2.893	0.004
TreatmentMedOT	-0.104	0.270	-0.383	0.702
TreatmentOTA	0.596	0.257	2.318	0.021
ObsNo	-0.122	0.049	-2.508	0.013
TestNo	-0.168	0.053	-3.146	0.002
PropPar	0.012	0.023	0.545	0.606
IOFLine	0.003	0.003	0.891	0.411
IOFVoc	-0.011	0.009	-1.276	0.253
JuvVoc	0.010	0.008	1.245	0.265
JuvLoc	-0.001	0.001	-0.452	0.670
TreatmentHighAVP:PropPar	0.050	0.020	2.504	0.013
TreatmentHighOT:PropPar	0.045	0.019	2.341	0.020
TreatmentLowAVP:PropPar	0.025	0.020	1.243	0.215
TreatmentLowOT:PropPar	-0.030	0.019	-1.599	0.111
TreatmentMedOT:PropPar	-0.012	0.021	-0.574	0.566
TreatmentOTA:PropPar	0.000	0.019	-0.005	0.996
TreatmentHighAVP:IOFLine	-0.003	0.003	-1.177	0.240
TreatmentHighOT:IOFLine	-0.007	0.003	-2.599	0.010
TreatmentLowAVP:IOFLine	-0.003	0.003	-1.270	0.205
TreatmentLowOT:IOFLine	-0.009	0.003	-3.584	< .001
TreatmentMedOT:IOFLine	-0.003	0.003	-1.096	0.274
TreatmentOTA:IOFLine	-0.001	0.003	-0.547	0.585
TreatmentHighAVP:IOFVoc	-0.017	0.008	-2.266	0.024
TreatmentHighOT:IOFVoc	0.003	0.007	0.430	0.668
TreatmentLowAVP:IOFVoc	-0.009	0.007	-1.226	0.221
TreatmentLowOT:IOFVoc	0.015	0.007	2.115	0.035
TreatmentMedOT:IOFVoc	0.004	0.007	0.520	0.604
TreatmentOTA:IOFVoc	-0.001	0.007	-0.071	0.943
TreatmentHighAVP:JuvVoc	0.024	0.007	3.364	< .001
TreatmentHighOT:JuvVoc	0.021	0.007	3.123	0.002
TreatmentLowAVP:JuvVoc	0.004	0.007	0.554	0.580

TreatmentLowOT:JuvVoc	-0.011	0.007	-1.563	0.119
TreatmentMedOT:JuvVoc	-0.007	0.007	-1.030	0.304
TreatmentOTA:JuvVoc	-0.003	0.007	-0.487	0.626
TreatmentHighAVP:JuvLoc	-0.002	0.001	-2.672	0.008
TreatmentHighOT:JuvLoc	0.001	0.001	1.000	0.318
TreatmentLowAVP:JuvLoc	0.000	0.001	0.418	0.676
TreatmentLowOT:JuvLoc	0.001	0.001	1.197	0.232
TreatmentMedOT:JuvLoc	0.002	0.001	2.120	0.035
TreatmentOTA:JuvLoc	-0.003	0.001	-3.070	0.002

4h. Zone Change Frequency

	β	SE	t-value	p
(Intercept)	12.940	0.711	18.205	< .001
TreatmentHighAVP	-1.502	0.564	-2.663	0.008
TreatmentHighOT	-0.792	0.564	-1.405	0.161
TreatmentLowAVP	-0.131	0.566	-0.232	0.817
TreatmentLowOT	-0.278	0.564	-0.492	0.623
TreatmentMedOT	-2.251	0.569	-3.958	< .001
TreatmentOTA	-1.032	0.564	-1.829	0.068
ObsNo	-0.233	0.107	-2.184	0.030
TestNo	-0.473	0.124	-3.829	< .001
PropPar	0.065	0.030	2.155	0.032
CnRPar	-0.027	0.039	-0.693	0.489
IOFProx	-0.039	0.023	-1.709	0.088
IOFGrate	0.145	0.029	5.064	< .001
JuvVoc	0.027	0.012	2.302	0.022
JuvLoc	0.008	0.002	4.034	< .001
TreatmentHighAVP:PropPar	-0.056	0.043	-1.312	0.190
TreatmentHighOT:PropPar	-0.027	0.043	-0.625	0.532
TreatmentLowAVP:PropPar	-0.022	0.043	-0.501	0.617
TreatmentLowOT:PropPar	0.033	0.043	0.760	0.448
TreatmentMedOT:PropPar	-0.001	0.043	-0.025	0.980
TreatmentOTA:PropPar	-0.031	0.043	-0.720	0.472
TreatmentHighAVP:CnRPar	0.071	0.053	1.337	0.182
TreatmentHighOT:CnRPar	0.037	0.057	0.652	0.515
TreatmentLowAVP:CnRPar	0.003	0.054	0.055	0.956
TreatmentLowOT:CnRPar	-0.080	0.053	-1.504	0.134
TreatmentMedOT:CnRPar	-0.074	0.055	-1.362	0.174
TreatmentOTA:CnRPar	-0.029	0.057	-0.506	0.614
TreatmentHighAVP:IOFProx	-0.004	0.032	-0.125	0.901

TreatmentHighOT:IOFProx	0.004	0.032	0.124	0.902
TreatmentLowAVP:IOFProx	-0.025	0.032	-0.771	0.441
TreatmentLowOT:IOFProx	-0.025	0.032	-0.795	0.427
TreatmentMedOT:IOFProx	0.037	0.033	1.143	0.254
TreatmentOTA:IOFProx	0.027	0.032	0.841	0.401
TreatmentHighAVP:IOFGrate	-0.053	0.040	-1.324	0.186
TreatmentHighOT:IOFGrate	-0.097	0.041	-2.403	0.017
TreatmentLowAVP:IOFGrate	-0.049	0.040	-1.228	0.220
TreatmentLowOT:IOFGrate	-0.045	0.041	-1.120	0.264
TreatmentMedOT:IOFGrate	-0.055	0.040	-1.366	0.173
TreatmentOTA:IOFGrate	-0.006	0.040	-0.146	0.884
TreatmentHighAVP:JuvVoc	0.009	0.017	0.518	0.605
TreatmentHighOT:JuvVoc	-0.010	0.018	-0.540	0.590
TreatmentLowAVP:JuvVoc	-0.013	0.016	-0.799	0.425
TreatmentLowOT:JuvVoc	-0.001	0.017	-0.046	0.963
TreatmentMedOT:JuvVoc	-0.009	0.017	-0.532	0.595
TreatmentOTA:JuvVoc	-0.023	0.017	-1.349	0.178
TreatmentHighAVP:JuvLoc	-0.002	0.003	-0.875	0.382
TreatmentHighOT:JuvLoc	-0.004	0.003	-1.340	0.181
TreatmentLowAVP:JuvLoc	0.000	0.003	-0.091	0.928
TreatmentLowOT:JuvLoc	-0.001	0.003	-0.291	0.772
TreatmentMedOT:JuvLoc	0.000	0.003	-0.020	0.984
TreatmentOTA:JuvLoc	-0.002	0.003	-0.629	0.530

4i. Parent-directed Lip-smacking

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	2.850	0.490	5.812	< .001
TreatmentHighAVP	-1.307	1.100	-1.188	0.235
TreatmentHighOT	1.383	0.575	2.406	0.016
TreatmentLowAVP	-0.882	0.577	-1.529	0.126
TreatmentLowOT	-0.863	0.817	-1.057	0.291
TreatmentMedOT	-1.359	0.715	-1.902	0.057
TreatmentOTA	1.105	0.965	1.146	0.252
ObsNo	-0.634	0.105	-6.028	< .001
TestNo	-0.605	0.116	-5.231	< .001
IOFProx	0.101	0.044	2.304	0.021
IOFVoc	0.037	0.011	3.435	< .001
TreatmentHighAVP:IOFProx	-0.121	0.054	-2.256	0.024
TreatmentHighOT:IOFProx	-0.092	0.046	-2.010	0.044

TreatmentLowAVP:IOFProx	-0.134	0.047	-2.852	0.004
TreatmentLowOT:IOFProx	-0.090	0.050	-1.790	0.073
TreatmentMedOT:IOFProx	-0.147	0.046	-3.174	0.002
TreatmentOTA:IOFProx	0.131	0.101	1.297	0.195
TreatmentHighAVP:IOFVoc	-0.019	0.011	-1.745	0.081
TreatmentHighOT:IOFVoc	-0.036	0.011	-3.224	0.001
TreatmentLowAVP:IOFVoc	-0.032	0.012	-2.723	0.006
TreatmentLowOT:IOFVoc	-0.013	0.011	-1.165	0.244
TreatmentMedOT:IOFVoc	-0.024	0.011	-2.175	0.030
TreatmentOTA:IOFVoc	0.010	0.019	0.524	0.600
Zero-part coefficients				
(Intercept)	-1.127	NaN	NaN	NA
TreatmentHighAVP	1.758	NaN	NaN	NA
TreatmentHighOT	-0.631	10.256	-0.062	0.951
TreatmentLowAVP	-1.144	16.348	-0.070	0.944
TreatmentLowOT	0.042	NaN	NaN	NA
TreatmentMedOT	-0.416	NaN	NaN	NA
TreatmentOTA	0.2520 8	55.030	0.000	1.000
ObsNo	1.150	0.640	1.799	0.072
TestNo	-2.044	2.670	-0.766	0.444
IOFProx	0.847	4.385	0.193	0.847
IOFVoc	0.203	0.411	0.493	0.622
TreatmentHighAVP:IOFProx	0.152	17.084	0.009	0.993
TreatmentHighOT:IOFProx	-0.203	4.127	-0.049	0.961
TreatmentLowAVP:IOFProx	-0.672	4.836	-0.139	0.889
TreatmentLowOT:IOFProx	-0.066	38.839	-0.002	0.999
TreatmentMedOT:IOFProx	-0.885	4.434	-0.200	0.842
TreatmentOTA:IOFProx	1.229	64.708	0.019	0.985
TreatmentHighAVP:IOFVoc	0.061	3.490	0.017	0.986
TreatmentHighOT:IOFVoc	-0.296	0.628	-0.472	0.637
TreatmentLowAVP:IOFVoc	-0.222	0.301	-0.739	0.460
TreatmentLowOT:IOFVoc	-0.066	12.744	-0.005	0.996
TreatmentMedOT:IOFVoc	-0.168	0.397	-0.423	0.672
TreatmentOTA:IOFVoc	-0.084	19.100	-0.004	0.996

4j. Stranger-directed Lip-smacking

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	1.920	0.565	3.398	< .001
TreatmentHighAVP	-0.452	0.474	-0.954	0.340

TreatmentHighOT	0.725	0.555	1.307	0.191
TreatmentLowAVP	-0.527	0.512	-1.029	0.304
TreatmentLowOT	-0.677	0.529	-1.278	0.201
TreatmentMedOT	0.110	0.522	0.211	0.833
TreatmentOTA	-1.102	0.756	-1.459	0.145
ObsNo	-0.547	0.046	-11.837	< .001
TestNo	0.058	0.045	1.291	0.197
PropPar	-0.004	0.016	-0.282	0.778
IOFProx	-0.041	0.014	-3.006	0.003
JuvLoc	0.004	0.005	0.771	0.441
TreatmentHighAVP:PropPar	0.028	0.013	2.193	0.028
TreatmentHighOT:PropPar	-0.016	0.018	-0.856	0.392
TreatmentLowAVP:PropPar	0.006	0.013	0.477	0.633
TreatmentLowOT:PropPar	0.040	0.015	2.725	0.006
TreatmentMedOT:PropPar	0.015	0.019	0.797	0.425
TreatmentOTA:PropPar	0.035	0.025	1.438	0.150
TreatmentHighAVP:IOFProx	0.015	0.014	1.072	0.284
TreatmentHighOT:IOFProx	0.008	0.012	0.661	0.509
TreatmentLowAVP:IOFProx	0.016	0.014	1.140	0.254
TreatmentLowOT:IOFProx	0.025	0.014	1.729	0.084
TreatmentMedOT:IOFProx	0.043	0.013	3.416	< .001
TreatmentOTA:IOFProx	0.017	0.015	1.081	0.280
TreatmentHighAVP:JuvLoc	-0.007	0.005	-1.347	0.178
TreatmentHighOT:JuvLoc	0.004	0.006	0.652	0.514
TreatmentLowAVP:JuvLoc	-0.009	0.005	-1.612	0.107
TreatmentLowOT:JuvLoc	-0.005	0.005	-0.995	0.320
TreatmentMedOT:JuvLoc	-0.005	0.005	-1.053	0.293
TreatmentOTA:JuvLoc	-0.014	0.007	-2.076	0.038
Zero-part coefficients				
(Intercept)	-1.234	1.285	-0.960	0.337
TreatmentHighAVP	-0.334	1.791	-0.187	0.852
TreatmentHighOT	-0.609	NaN	NaN	NA
TreatmentLowAVP	-0.094	1.764	-0.053	0.958
TreatmentLowOT	0.146	0.518	0.282	0.778
TreatmentMedOT	0.490	1.073	0.457	0.648
TreatmentOTA	0.663	1.821	0.364	0.716
ObsNo	0.206	0.158	1.306	0.191
TestNo	0.022	0.178	0.125	0.901
PropPar	-0.037	0.033	-1.144	0.253
IOFProx	-0.016	0.032	-0.490	0.624
JuvLoc	0.005	0.004	1.506	0.132

TreatmentHighAVP:PropPar	0.081	0.043	1.901	0.057
TreatmentHighOT:PropPar	-1.011	NaN	NaN	NA
TreatmentLowAVP:PropPar	0.010	0.040	0.247	0.805
TreatmentLowOT:PropPar	0.048	NaN	NaN	NA
TreatmentMedOT:PropPar	-0.040	0.046	-0.886	0.375
TreatmentOTA:PropPar	-0.077	0.059	-1.290	0.197
TreatmentHighAVP:IOFProx	-0.019	0.047	-0.398	0.691
TreatmentHighOT:IOFProx	0.346	NaN	NaN	NA
TreatmentLowAVP:IOFProx	0.051	0.040	1.256	0.209
TreatmentLowOT:IOFProx	0.112	0.051	2.172	0.030
TreatmentMedOT:IOFProx	0.085	0.044	1.939	0.053
TreatmentOTA:IOFProx	0.094	0.045	2.095	0.036
TreatmentHighAVP:JuvLoc	-0.015	0.015	-0.986	0.324
TreatmentHighOT:JuvLoc	0.119	NaN	NaN	NA
TreatmentLowAVP:JuvLoc	-0.011	0.014	-0.775	0.439
TreatmentLowOT:JuvLoc	-0.006	0.005	-1.240	0.215
TreatmentMedOT:JuvLoc	-0.004	0.005	-0.876	0.381
TreatmentOTA:JuvLoc	0.000	0.014	-0.028	0.977

4k. Back-arching/Tail-lashing

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	1.807	0.416	4.343	< .001
TreatmentHighAVP	-0.645	0.424	-1.521	0.128
TreatmentHighOT	1.276	0.311	4.106	< .001
TreatmentLowAVP	-0.871	0.429	-2.033	0.042
TreatmentLowOT	-0.308	0.319	-0.966	0.334
TreatmentMedOT	0.639	0.401	1.593	0.111
TreatmentOTA	3.111	3.938	0.790	0.430
ObsNo	-0.654	0.044	-14.823	< .001
PropPar	0.000	0.022	-0.013	0.990
IOFProx	0.009	0.018	0.511	0.610
IOFVoc	0.012	0.006	1.914	0.056
TreatmentHighAVP:PropPar	0.020	0.026	0.754	0.451
TreatmentHighOT:PropPar	-0.021	0.019	-1.103	0.270
TreatmentLowAVP:PropPar	0.057	0.016	3.632	< .001
TreatmentLowOT:PropPar	0.078	0.017	4.615	< .001
TreatmentMedOT:PropPar	-0.006	0.023	-0.261	0.794
TreatmentOTA:PropPar	-0.206	0.253	-0.816	0.415
TreatmentHighAVP:IOFProx	0.007	0.028	0.238	0.812

TreatmentHighOT:IOFProx	-0.040	0.012	-3.389	< .001
TreatmentLowAVP:IOFProx	-0.107	0.022	-4.834	< .001
TreatmentLowOT:IOFProx	-0.027	0.015	-1.739	0.082
TreatmentMedOT:IOFProx	-0.013	0.014	-0.926	0.355
TreatmentOTA:IOFProx	-0.074	0.063	-1.172	0.241
TreatmentHighAVP:IOFVoc	0.002	0.008	0.180	0.857
TreatmentHighOT:IOFVoc	-0.010	0.004	-2.708	0.007
TreatmentLowAVP:IOFVoc	-0.021	0.005	-4.202	< .001
TreatmentLowOT:IOFVoc	-0.018	0.004	-3.976	< .001
TreatmentMedOT:IOFVoc	-0.007	0.004	-1.520	0.129
TreatmentOTA:IOFVoc	-0.010	0.024	-0.395	0.693
Zero-part coefficients				
(Intercept)	-2.113	0.968	-2.183	0.029
TreatmentHighAVP	0.218	0.933	0.233	0.815
TreatmentHighOT	-0.751	0.961	-0.782	0.434
TreatmentLowAVP	-1.036	1.415	-0.732	0.464
TreatmentLowOT	-1.116	1.747	-0.639	0.523
TreatmentMedOT	0.689	0.787	0.875	0.381
TreatmentOTA	3.494	2.703	1.293	0.196
ObsNo	0.605	0.197	3.075	0.002
TestNo	0.197	0.189	1.042	0.298
PropPar	-0.013	0.024	-0.558	0.577
IOFProx	0.052	0.030	1.720	0.085
IOFVoc	-0.009	0.009	-0.986	0.324
TreatmentHighAVP:PropPar	-0.056	0.048	-1.157	0.247
TreatmentHighOT:PropPar	-0.048	0.038	-1.269	0.205
TreatmentLowAVP:PropPar	0.055	0.045	1.223	0.221
TreatmentLowOT:PropPar	0.154	0.114	1.350	0.177
TreatmentMedOT:PropPar	-0.014	0.037	-0.379	0.704
TreatmentOTA:PropPar	-0.317	0.173	-1.830	0.067
TreatmentHighAVP:IOFProx	0.049	0.064	0.764	0.445
TreatmentHighOT:IOFProx	-0.043	0.037	-1.152	0.250
TreatmentLowAVP:IOFProx	-0.082	0.065	-1.269	0.205
TreatmentLowOT:IOFProx	-0.047	0.045	-1.062	0.288
TreatmentMedOT:IOFProx	-0.019	0.036	-0.535	0.593
TreatmentOTA:IOFProx	0.071	0.087	0.808	0.419
TreatmentHighAVP:IOFVoc	0.026	0.021	1.247	0.212
TreatmentHighOT:IOFVoc	0.006	0.012	0.478	0.633
TreatmentLowAVP:IOFVoc	-0.007	0.016	-0.412	0.680
TreatmentLowOT:IOFVoc	-0.021	0.014	-1.483	0.138
TreatmentMedOT:IOFVoc	-0.006	0.013	-0.471	0.638

TreatmentOTA:IOFVoc	0.047	0.028	1.673	0.094

4l. Stranger Receive Aggression

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	-1.261	0.670	-1.881	0.060
TreatmentHighAVP	-4.251	2.094	-2.030	0.042
TreatmentHighOT	-2.387	1.532	-1.558	0.119
TreatmentLowAVP	0.681	0.424	1.605	0.109
TreatmentLowOT	0.512	0.467	1.097	0.273
TreatmentMedOT	-1.506	0.774	-1.947	0.052
TreatmentOTA	-4.958	3.935	-1.260	0.208
TestNo	0.294	0.118	2.481	0.013
JuvReun	-0.076	0.019	-4.035	< .001
IOFLine	-0.010	0.003	-3.112	0.002
IOFVoc	-0.003	0.005	-0.632	0.527
JuvVoc	0.007	0.003	2.294	0.022
TreatmentHighAVP:JuvReun	0.205	0.067	3.055	0.002
TreatmentHighOT:JuvReun	-0.022	0.086	-0.253	0.800
TreatmentLowAVP:JuvReun	0.025	0.019	1.365	0.172
TreatmentLowOT:JuvReun	0.050	0.025	2.020	0.043
TreatmentMedOT:JuvReun	0.115	0.032	3.660	< .001
TreatmentOTA:JuvReun	0.156	0.120	1.295	0.195
TreatmentHighAVP:IOFLine	-0.019	0.009	-2.046	0.041
TreatmentHighOT:IOFLine	0.009	0.005	1.626	0.104
TreatmentLowAVP:IOFLine	0.003	0.004	0.688	0.492
TreatmentLowOT:IOFLine	0.007	0.004	1.906	0.057
TreatmentMedOT:IOFLine	0.004	0.005	0.870	0.384
TreatmentOTA:IOFLine	-0.016	0.017	-0.954	0.340
TreatmentHighAVP:IOFVoc	0.044	0.015	3.022	0.003
TreatmentHighOT:IOFVoc	-0.008	0.009	-0.872	0.383
TreatmentLowAVP:IOFVoc	0.019	0.007	2.572	0.010
TreatmentLowOT:IOFVoc	0.004	0.005	0.745	0.456
TreatmentMedOT:IOFVoc	0.017	0.007	2.376	0.018
TreatmentOTA:IOFVoc	0.040	0.025	1.615	0.106
TreatmentHighAVP:JuvVoc	0.084	0.039	2.169	0.030
TreatmentHighOT:JuvVoc	0.000	0.009	0.019	0.985
TreatmentLowAVP:JuvVoc	-0.020	0.007	-2.965	0.003
TreatmentLowOT:JuvVoc	-0.002	0.006	-0.375	0.707
TreatmentMedOT:JuvVoc	0.024	0.014	1.732	0.083

TreatmentOTA:JuvVoc	0.072	0.073	0.990	0.322
Zero-part coefficients				
(Intercept)	-0.103	0.435	-0.238	0.812

Table 5. Overview of interactions between treatments and relationship quality variables. A ↑ indicates a positive relation between the relationship quality variable (in the columns) and treatment (in the rows) for that particular preference test outcome. A ↓ indicates a negative relation between the relationship quality variable and treatment. OT = Oxytocin; AVP = Arginine-vasopressin; OTA = Oxytocin Receptor Antagonist; JuvReun = *Juvenile Proximity;* PropPar = *Juvenile Parent Preference;* CnRPar = *Juvenile Parent Choice;* InfCarry = *Infant Proximity;* IOFProx = *IOF Proximity;* IOFGrate = *IOF Grate;* ParAff = *Parent Affiliation;* IOFLine = *IOF Locomotion;* IOFVoc = *IOF Vocalization;* JuvVoc = *Juvenile Vocalization;* JuvLoc = *Juvenile Locomotion*

	Juvenile Proximity Maintenance			**Infant Proximity Maintenance**			**Parents Affiliation**	**Infant Separation Distress**		**Juvenile Separation Distress**	
Table 5a.	**Zone Ratio Score**										
	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT											
Medium OT											↓
High OT		↑									
Low AVP		↑								↑	
High AVP											
OTA											
Table 5b.	**Percent Social Score**										
	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT			↓				↑				
Medium OT											
High OT		↑									
Low AVP		↑									
High AVP		↑									
OTA		↓	↑								

Table 5c. Parent Preference Zone Duration

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT											
Medium OT					↑					↑	
High OT		↑									
Low AVP		↑			↑						
High AVP	↑	↑								↑	
OTA		↑									

Table 5d. Stranger Preference Zone Duration

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT				↑	↓	↓	↑				
Medium OT		↓	↓								
High OT	↓	↓		↓	↓	↓	↓	↑			
Low AVP		↑	↑	↑		↑	↑	↓	↓		
High AVP	↑	↓	↑		↑	↑	↓	↓	↓		
OTA			↑		↑	↑	↓	↑			

Table 5e. Other Preference Zone Duration

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT				↓							
Medium OT											
High OT											
Low AVP											
High AVP											
OTA				↑							

Table 5f. Parent Grate Touch Duration

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT			↓								
Medium OT											
High OT	↑									↓	
Low AVP		↑			↑	↑		↑			
High AVP										↑	
OTA	↓		↑								

Table 5g. Stranger Grate Touch Duration

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT								↓	↓		
Medium OT											↑
High OT		↑						↑		↑	
Low AVP											
High AVP		↓							↓	↑	↓
OTA											↓

Table 5h. Change Zone Frequency

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT											
Medium OT											
High OT						↑					
Low AVP											
High AVP											
OTA											

Table 5i. Parent-directed lip-smacking

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT											
Medium OT					↓				↑		
High OT					↓				↓		
Low AVP					↓				↑		
High AVP					↑				↑		
OTA											

Table 5j. Stranger-direced lip-smacking

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT		↑									
Medium OT					↓						
High OT											
Low AVP											
High AVP											
OTA											↓

Table 5k. Back-arching/Tail-lashing

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT		↑							↑		
Medium OT											
High OT					↓				↑		
Low AVP		↑			↓				↑		
High AVP											
OTA											

Table 5l. Receive Aggression from Stranger Pair

	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	IOFLine	IOFVoc	JuvVoc	JuvLoc
Low OT	↓										
Medium OT	↑								↑		
High OT											
Low AVP									↑		↓
High AVP	↓							↑	↑		↑
OTA											

FIGURES

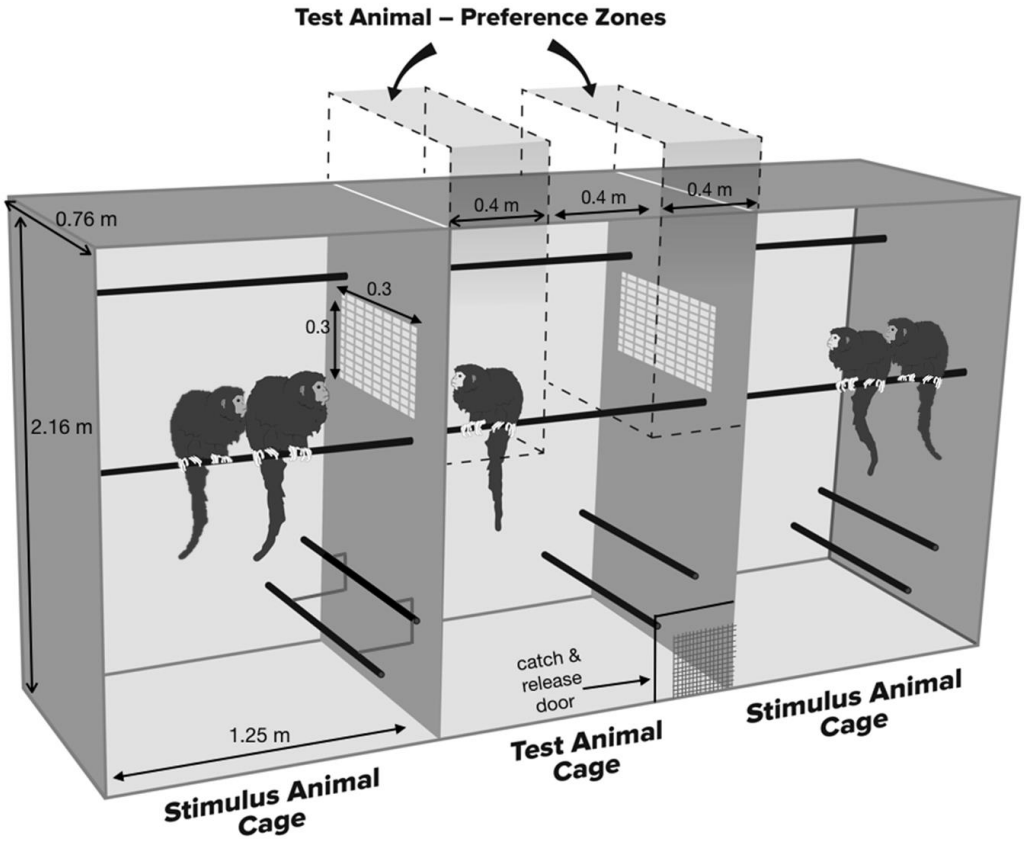


Figure 1. Schematic for parent preference test.

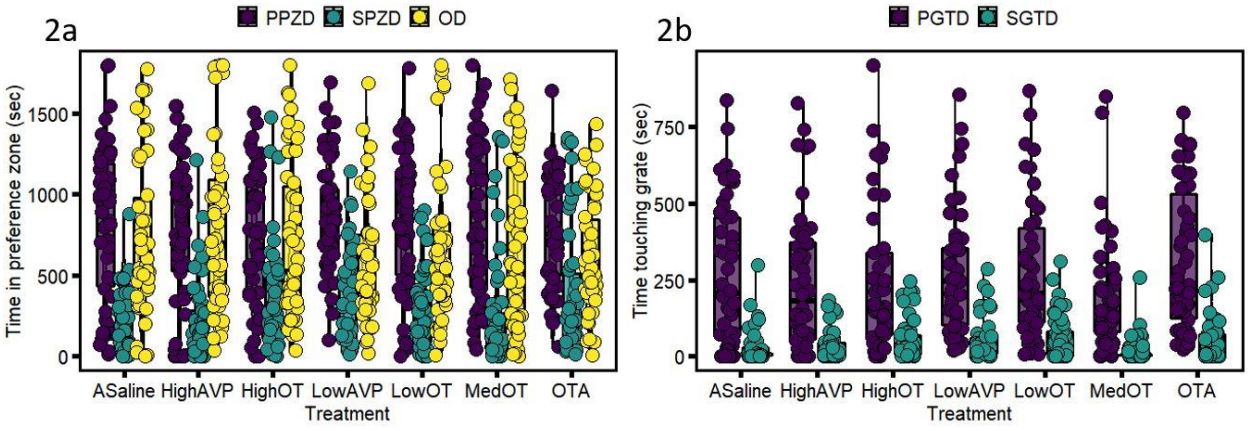


Figure 2. Summary of time females spend a) in preference zones and b) touching stimulus grates separated by *Treatment*. PPZD = Parent preference zone duration; SPZD = Stranger preference zone duration; OD = Other (or "non-social") zone duration; PGTD = Parent grate touch duration;

SGTD = Stranger preference zone duration; OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

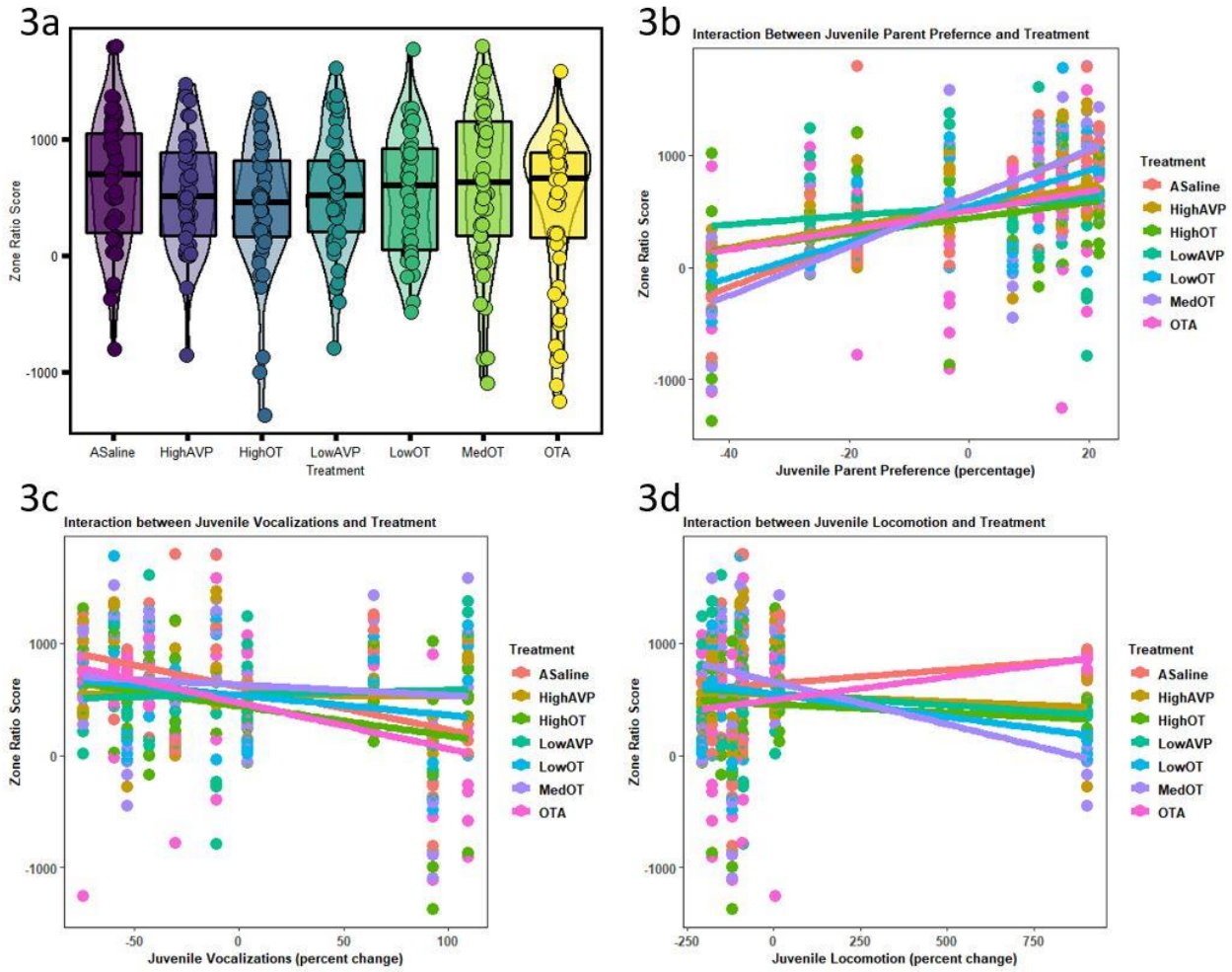


Figure 3. Zone Ratio score: a) the main effect of *Treatment*, interaction effects between *Treatment* and b) *Juvenile Parent Preference*, c) *Juvenile Vocalization*, and d) *Juvenile Locmotion.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

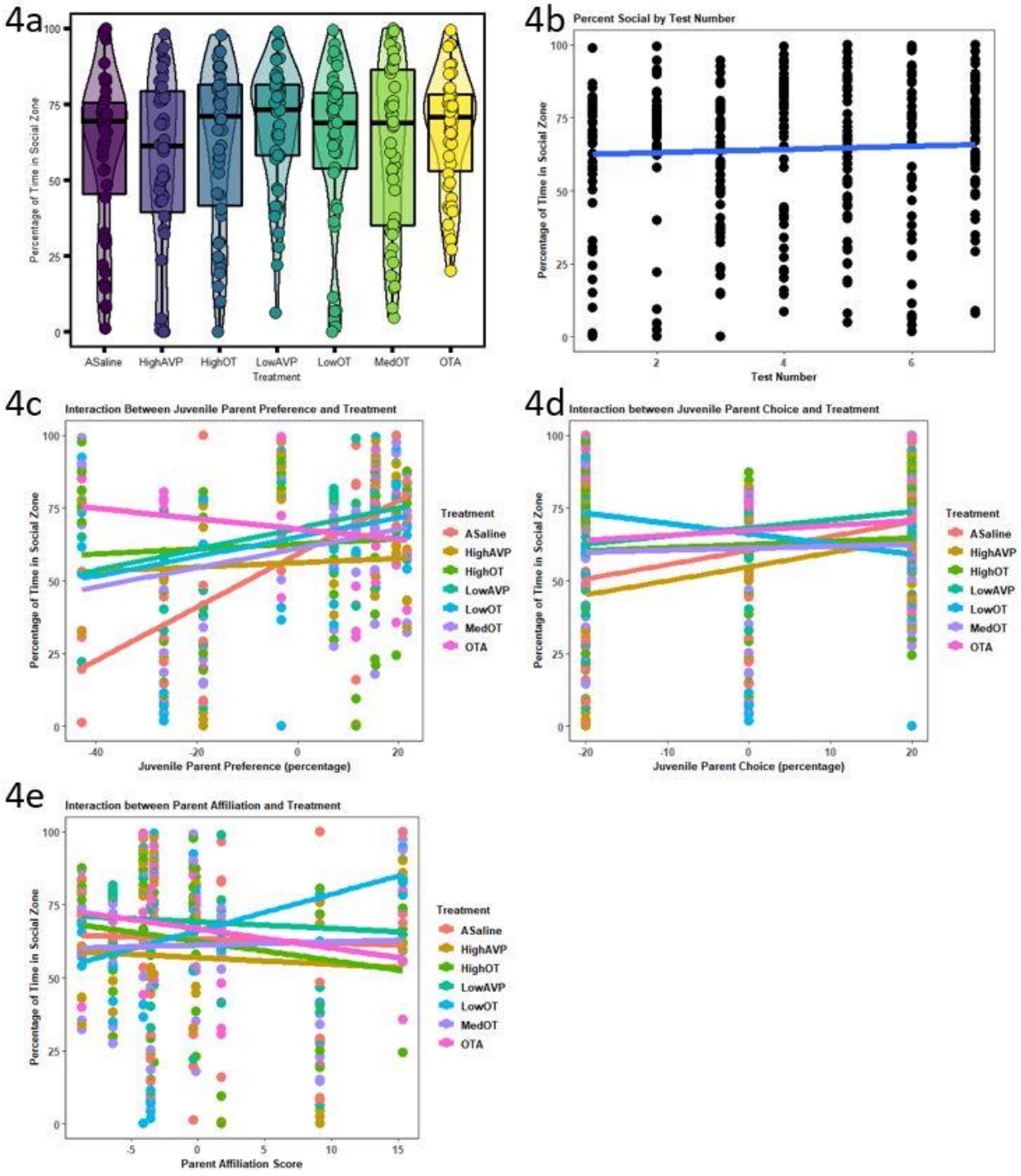
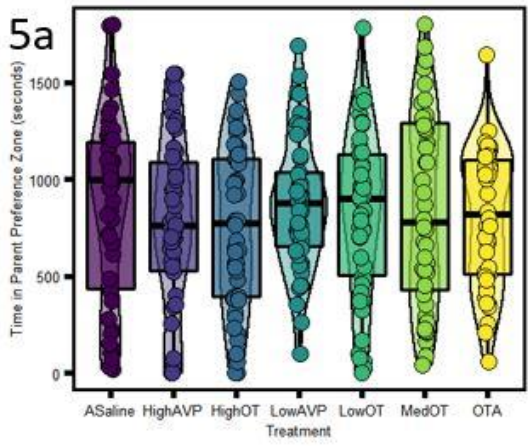
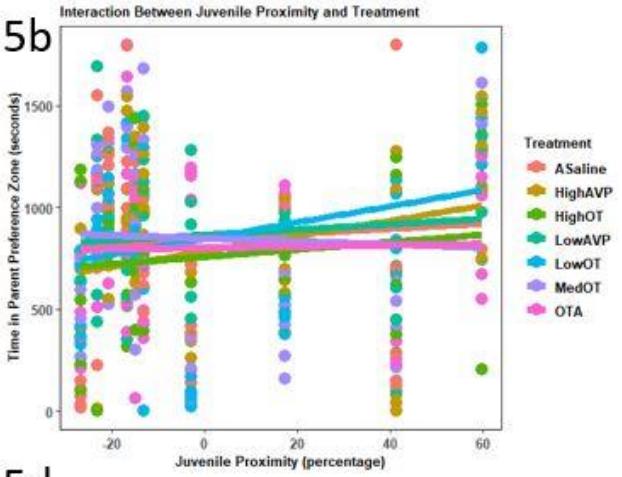
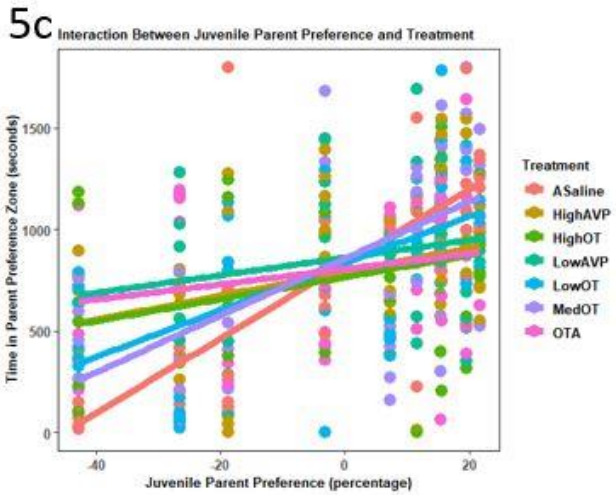


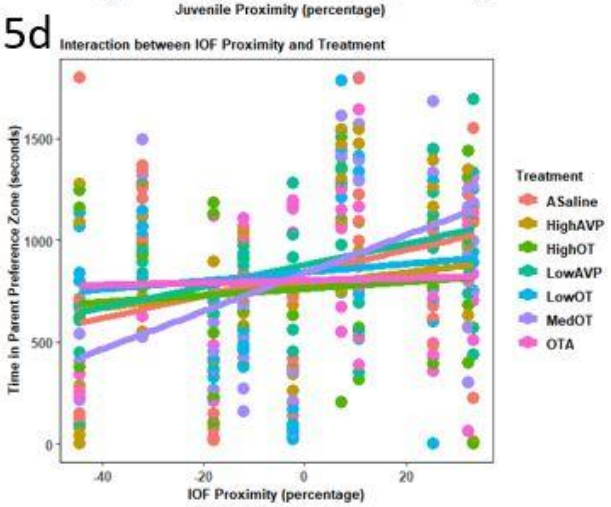
Figure 4. Percent Social score: a) the main effect of *Treatment*, b) *Test Number*, interaction effects between *Treatment* and c) *Juvenile Parent Preference*, d) *Juvenile Parent Choice*, and e) *Parent Affiliation.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist;

ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA









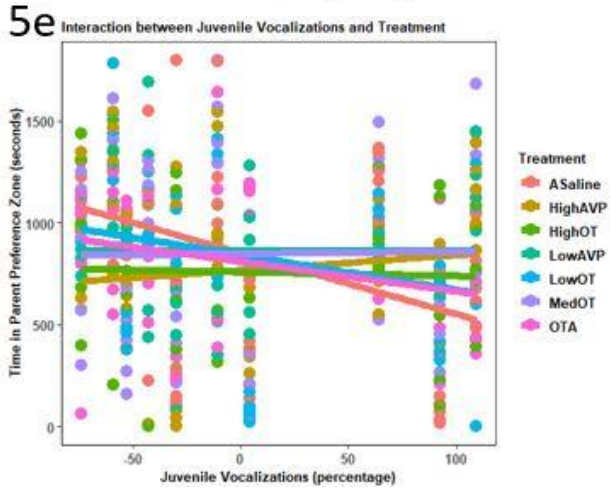
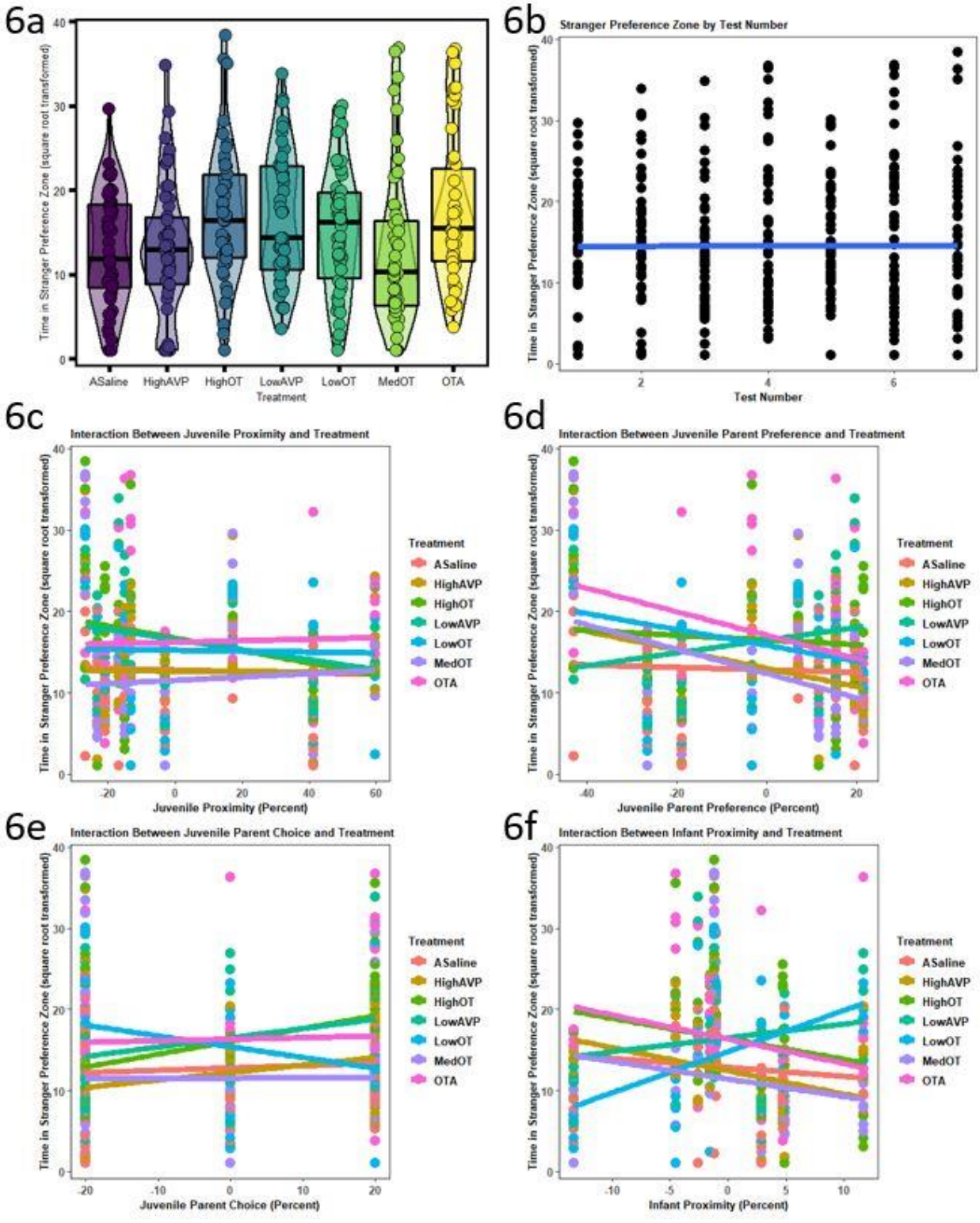


Figure 5. Parent Zone duration: a) the main effect of *Treatment*, interaction effects between *Treatment* and b) *Juvenile Proximity*, c) *Juvenile Parent Preference*, d) *IOF Proximity*, and e) *Juvenile Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA


6a
Time in Stranger Preference Zone (square root transformed)
ASaline
HighAVP
HighOT
LowAVP
LowOT
MedOT
OTA
Treatment
6b
Stranger Preference Zone by Test Number
Time in Stranger Preference Zone (square root transformed)
Test Number
6c
Interaction Between Juvenile Proximity and Treatment
Time in Stranger Preference Zone (square root transformed)
Juvenile Proximity (Percent)
Treatment
ASaline
HighAVP
HighOT
LowAVP
LowOT
MedOT
OTA
6d
Interaction Between Juvenile Parent Preference and Treatment
Time in Stranger Preference Zone (square root transformed)
Juvenile Parent Preference (Percent)
Treatment
ASaline
HighAVP
HighOT
LowAVP
LowOT
MedOT
OTA
6e
Interaction Between Juvenile Parent Choice and Treatment
Time in Stranger Preference Zone (square root transformed)
Juvenile Parent Choice (Percent)
Treatment
ASaline
HighAVP
HighOT
LowAVP
LowOT
MedOT
OTA
6f
Interaction Between Infant Proximity and Treatment
Time in Stranger Preference Zone (square root transformed)
Infant Proximity (Percent)
Treatment
ASaline
HighAVP
HighOT
LowAVP
LowOT
MedOT
OTA

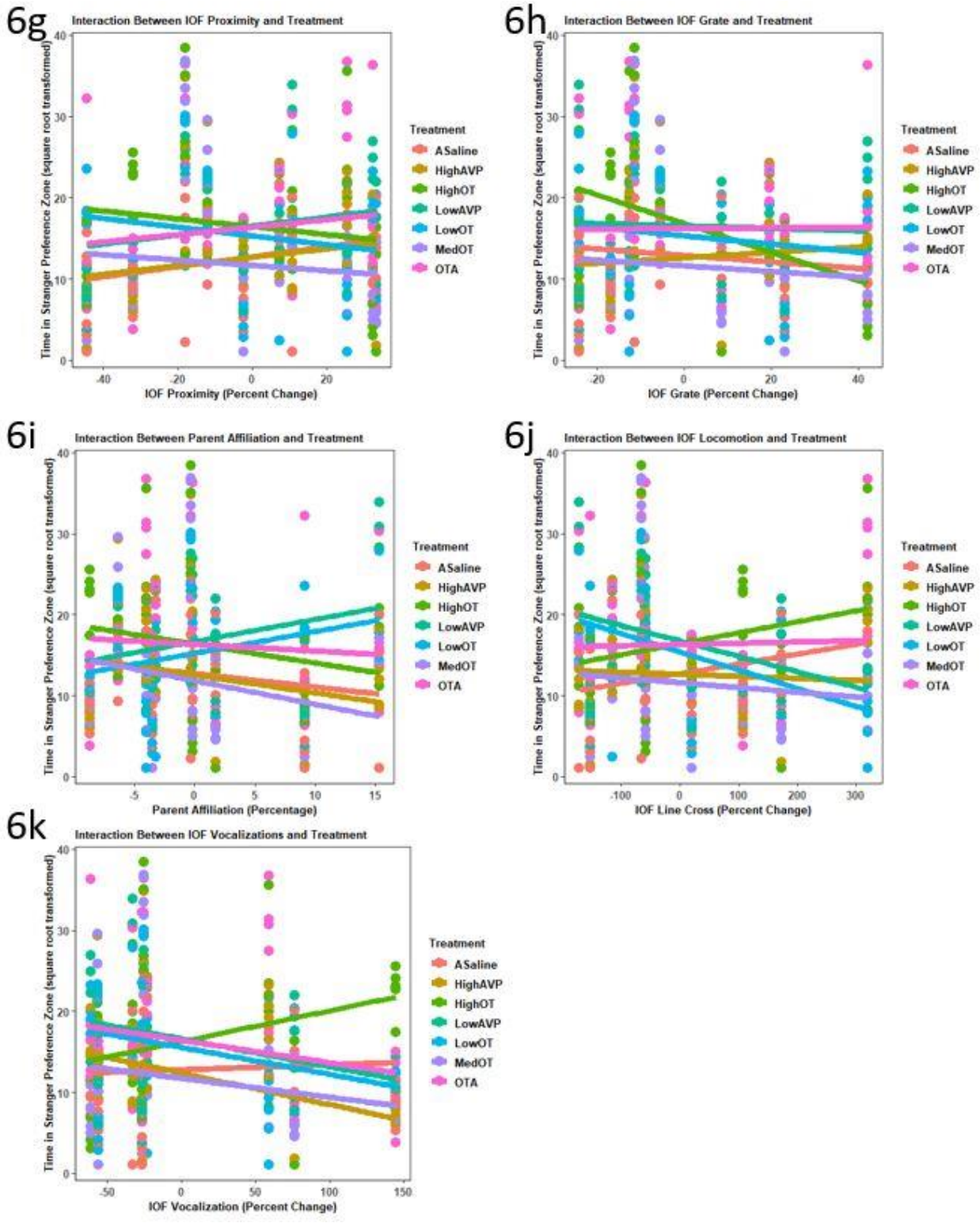


Figure 6. Stranger Zone duration: a) the main effect of *Treatment*, b) *Test Number*, interaction effects between *Treatment* and c) *Juvenile Proximity*, d) *Juvenile Parent Preference*, e) *Juvenile*

Parent Choice, f) *Infant Proximity*, g) *IOF Proximity*, h) *IOF Grate*, i) *Parent Affiliation*, j) *IOF Locomotion*, and k) *IOF Vocalizations*. OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

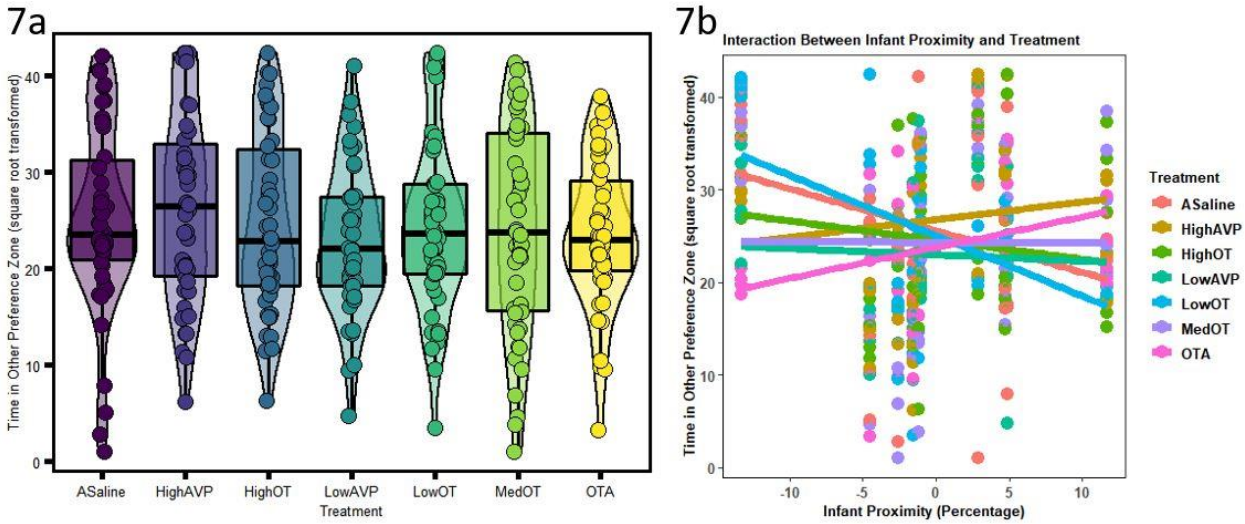
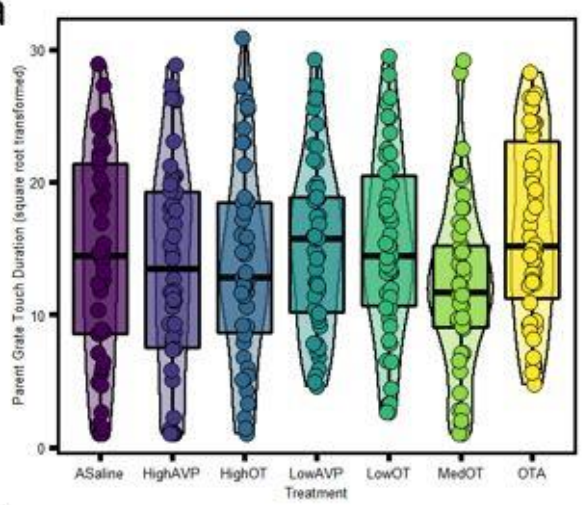
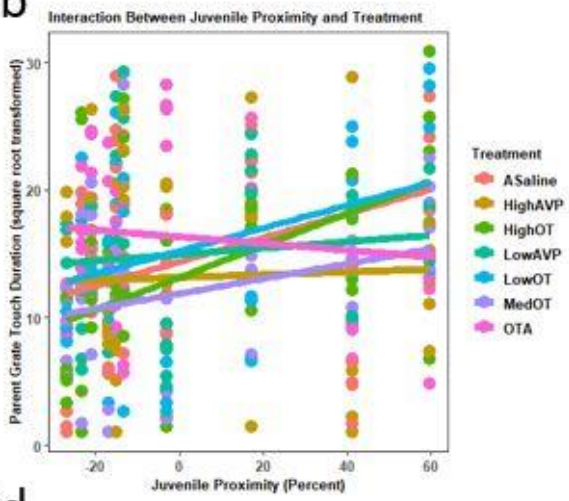


Figure 7. Other Zone duration: a) the main effect of *Treatment* and interaction effect between *Treatment* and b) *Infant Proximity*. OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

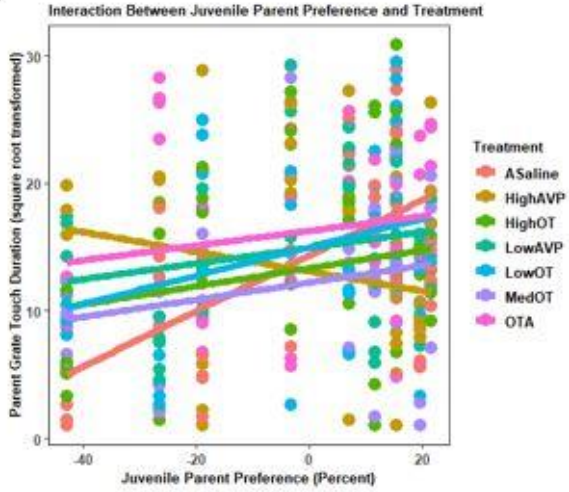
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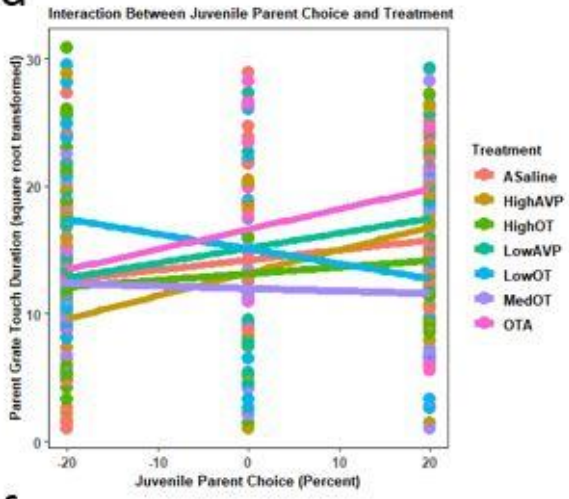
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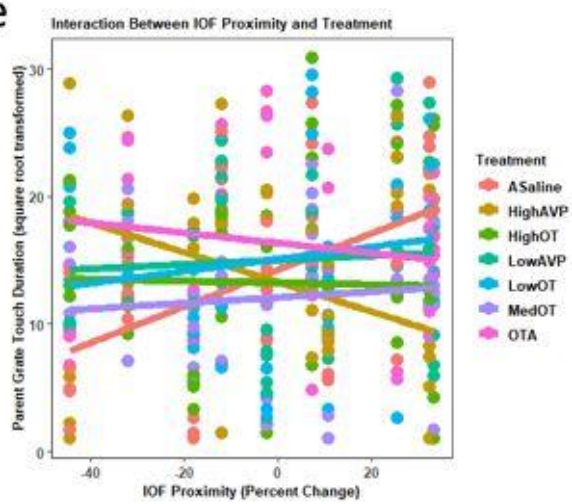
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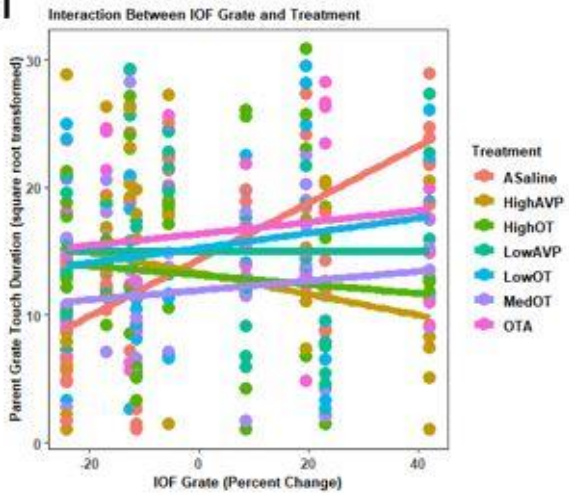
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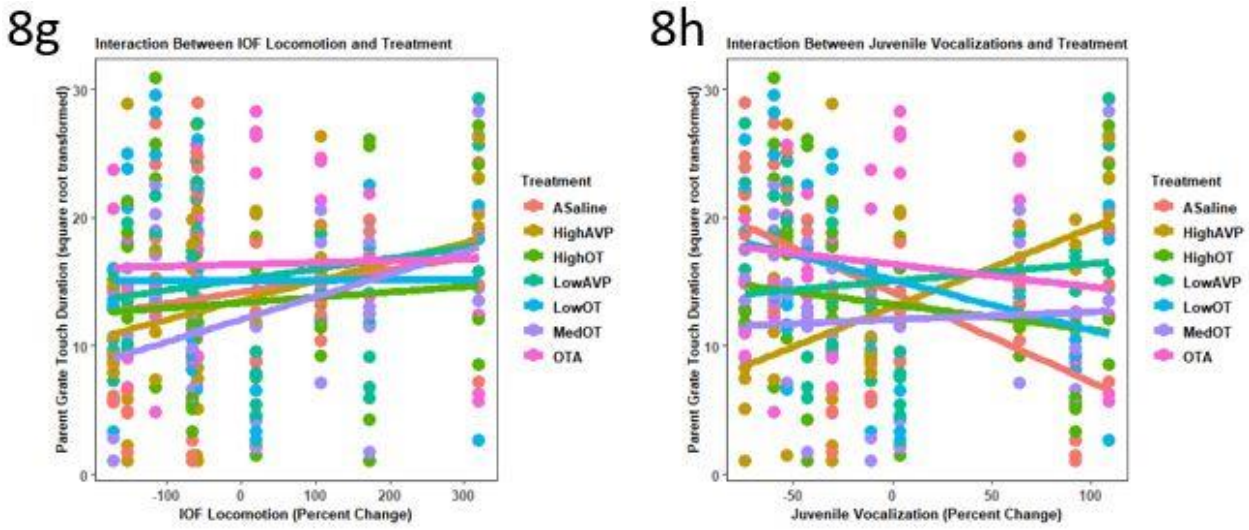


Figure 8. Parent Grate Touch duration: a) the main effect of *Treatment*, interaction effects between *Treatment* and b) *Juvenile Proximity*, c) *Juvenile Parent Preference*, d) *Juvenile Parent Choice*, e) *IOF Proximity*, f) *IOF Grate*, g) *IOF Locomotion*, and h) *Juvenile Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

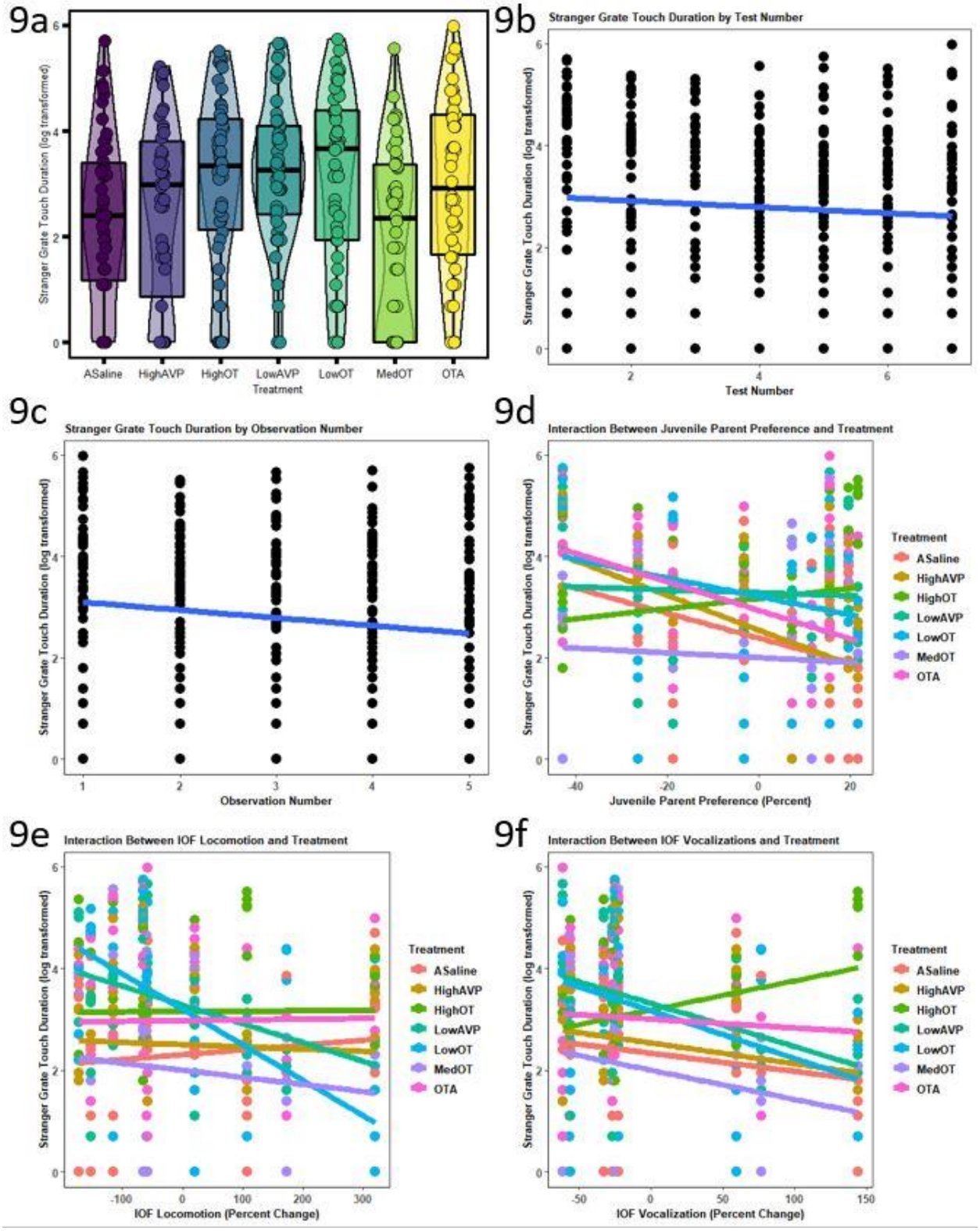
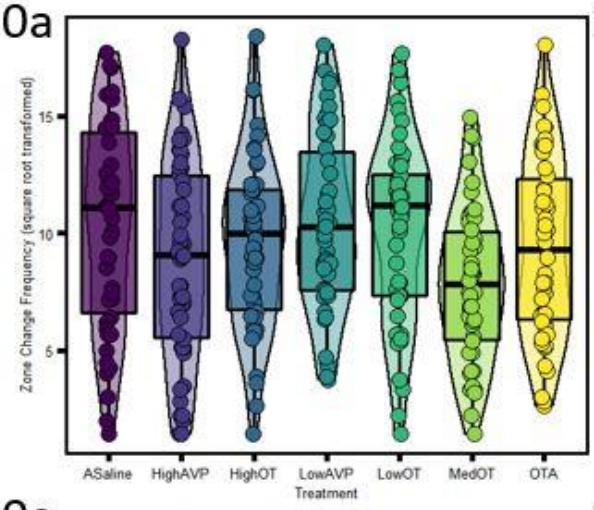


Figure 9. Stranger Grate Touch duration: a) the main effect of *Treatment*, b) *Test Number*, c) *Observation Number*, interaction effects between *Treatment* and d) *Juvenile Parent Preference*,

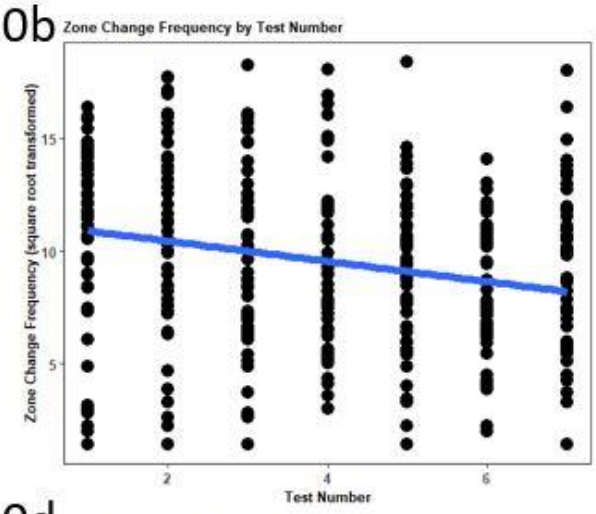
e) *IOF Locomotion,* f) *IOF Vocalizations,* g) *Juvenile Vocalization*, and h) *Juvenile Locomotion.*

OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

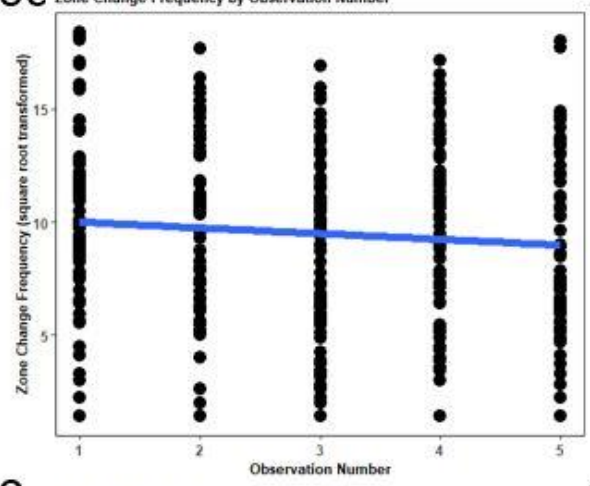
10a



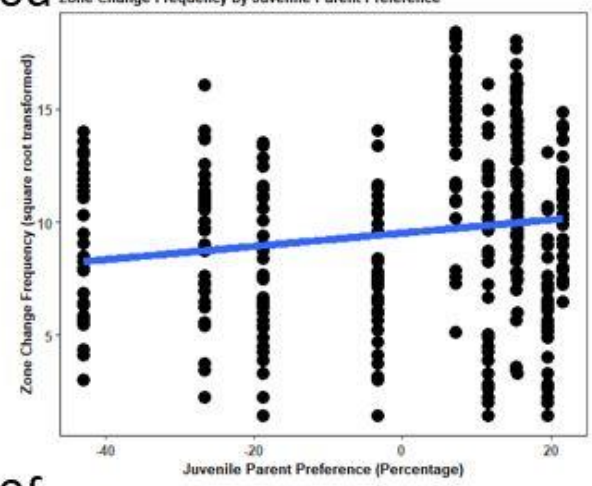
10b



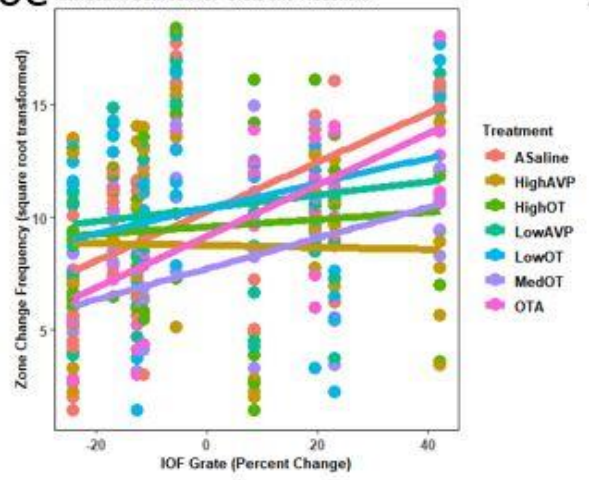
10c



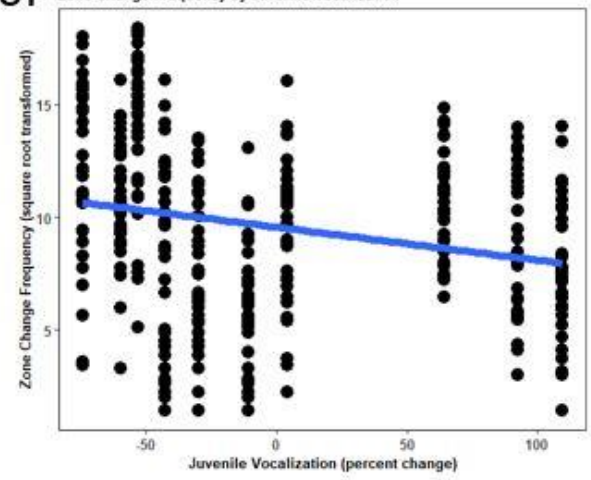
10d



10e



10f



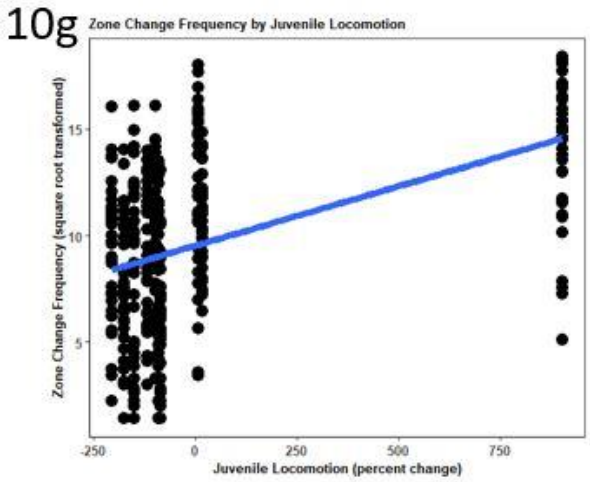


Figure 10. Zone Change frequency: a) the main effect of *Treatment*, b) *Test Number*, c) *Observation Number*, d) *Juvenile Parent Preference*, e) interaction effect between *Treatment* and *IOF Grate*, main effect of f) *Juvenile Vocalizations*, and g) *Juvenile Locomotion.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

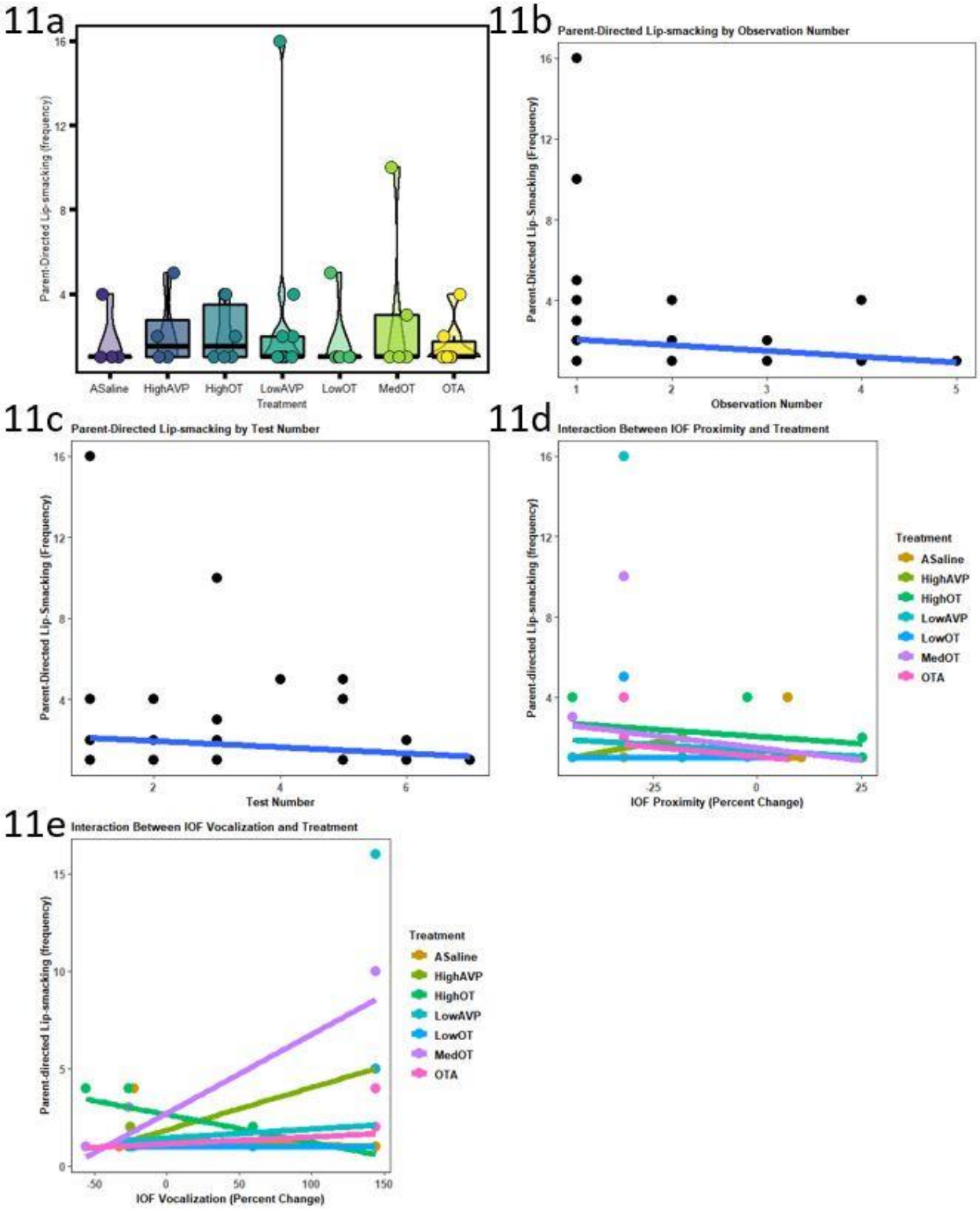


Figure 11. Parent-directed Lip-smacking frequency: a) the main effect of *Treatment*, b) *Observation Number*, c) *Test Number*, interaction effects between *Treatment* and d) *IOF*

Proximity, and e) *IOF Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

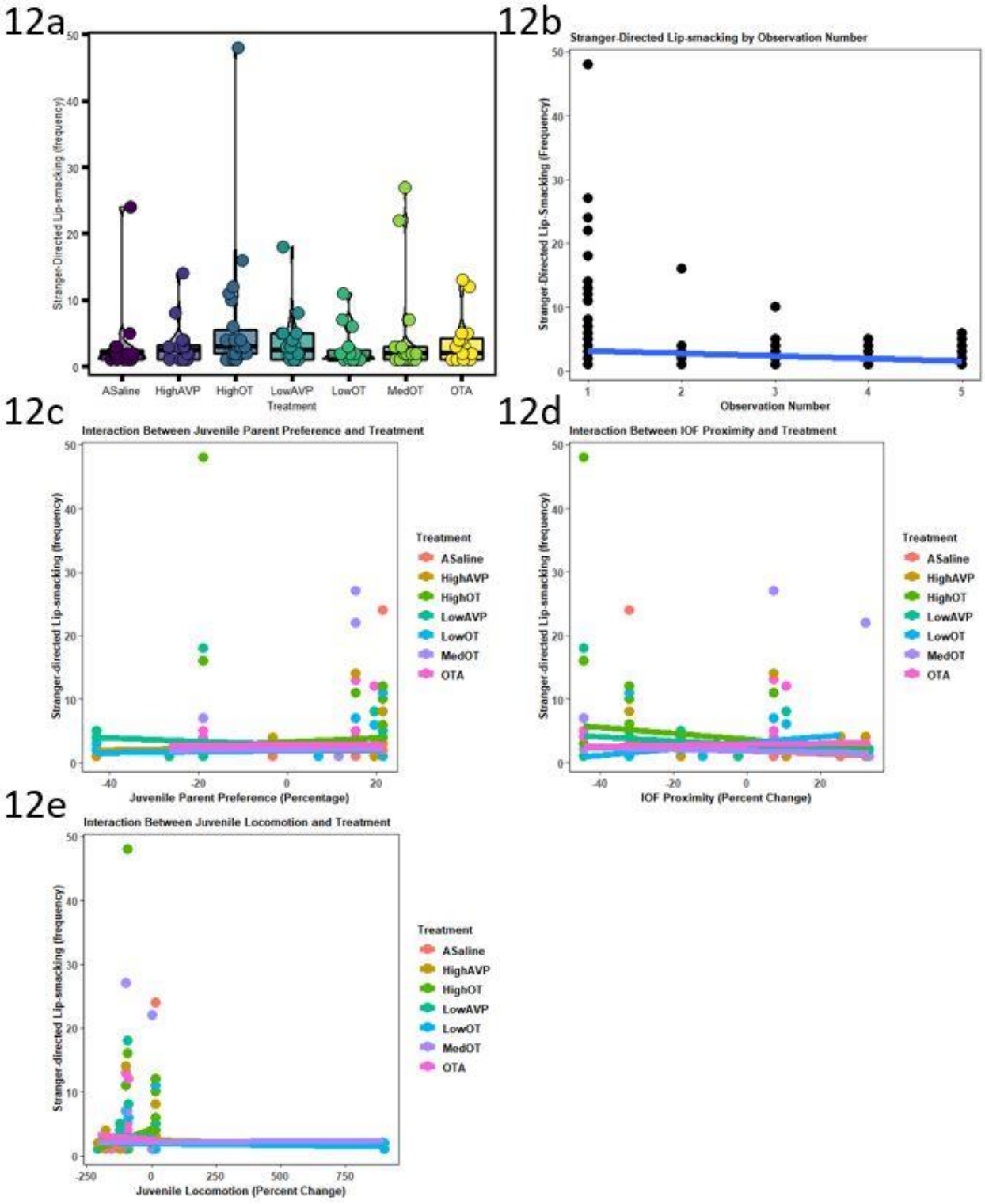


Figure 12. Stranger-directed Lip-smacking frequency: a) the main effect of *Treatment*, b) *Observation Number*, interaction effects between *Treatment* and c) *Juvenile Parent Preference*,

d) *IOF Proximity*, and e) *Juvenile Locomotion.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

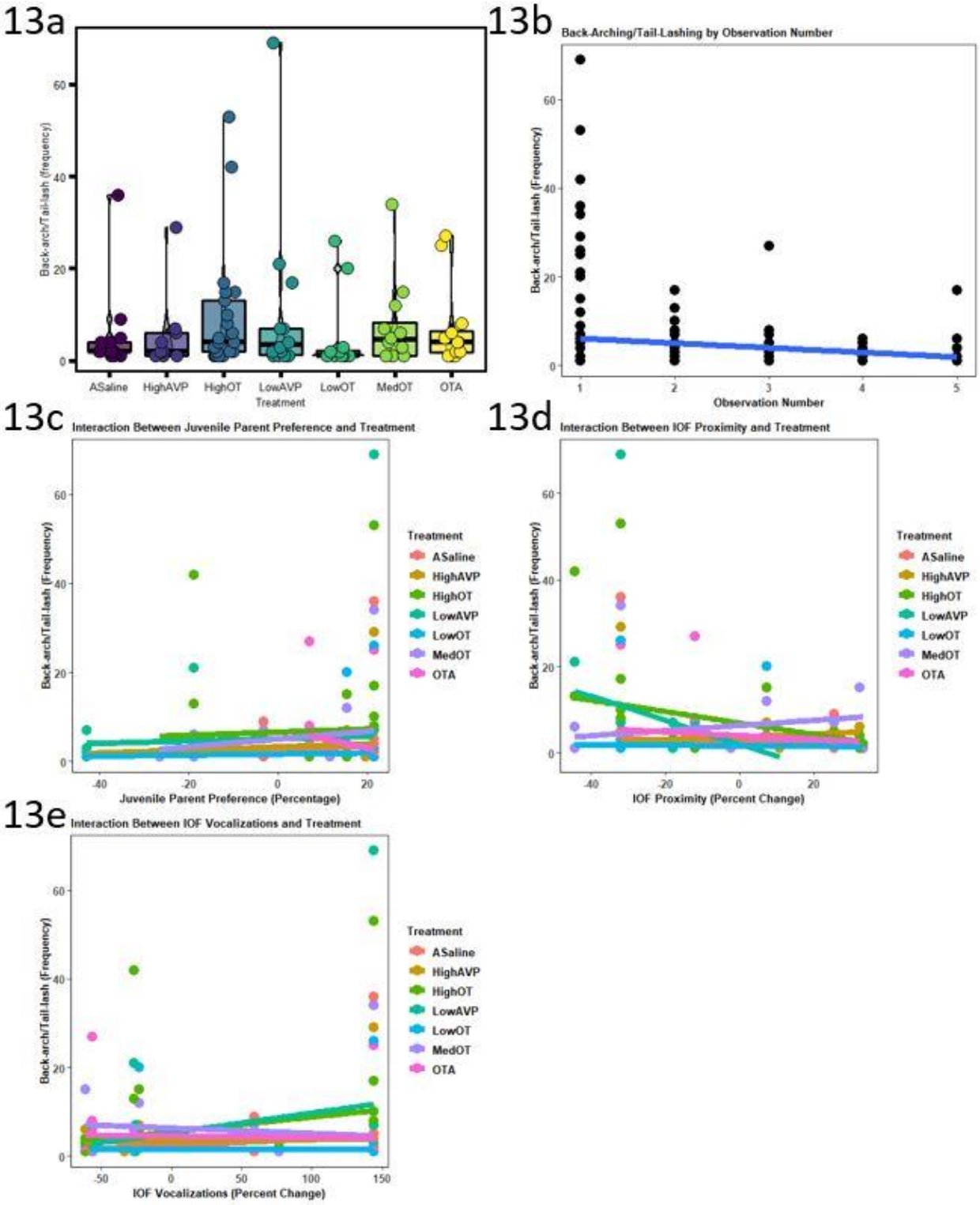


Figure 13. Back-arching/Tail-lashing frequency: a) the main effect of *Treatment*, b) *Observation Number*, interaction effects between *Treatment* and c) *Juvenile Parent Preference*, d) *IOF*

Proximity, and e) *IOF Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

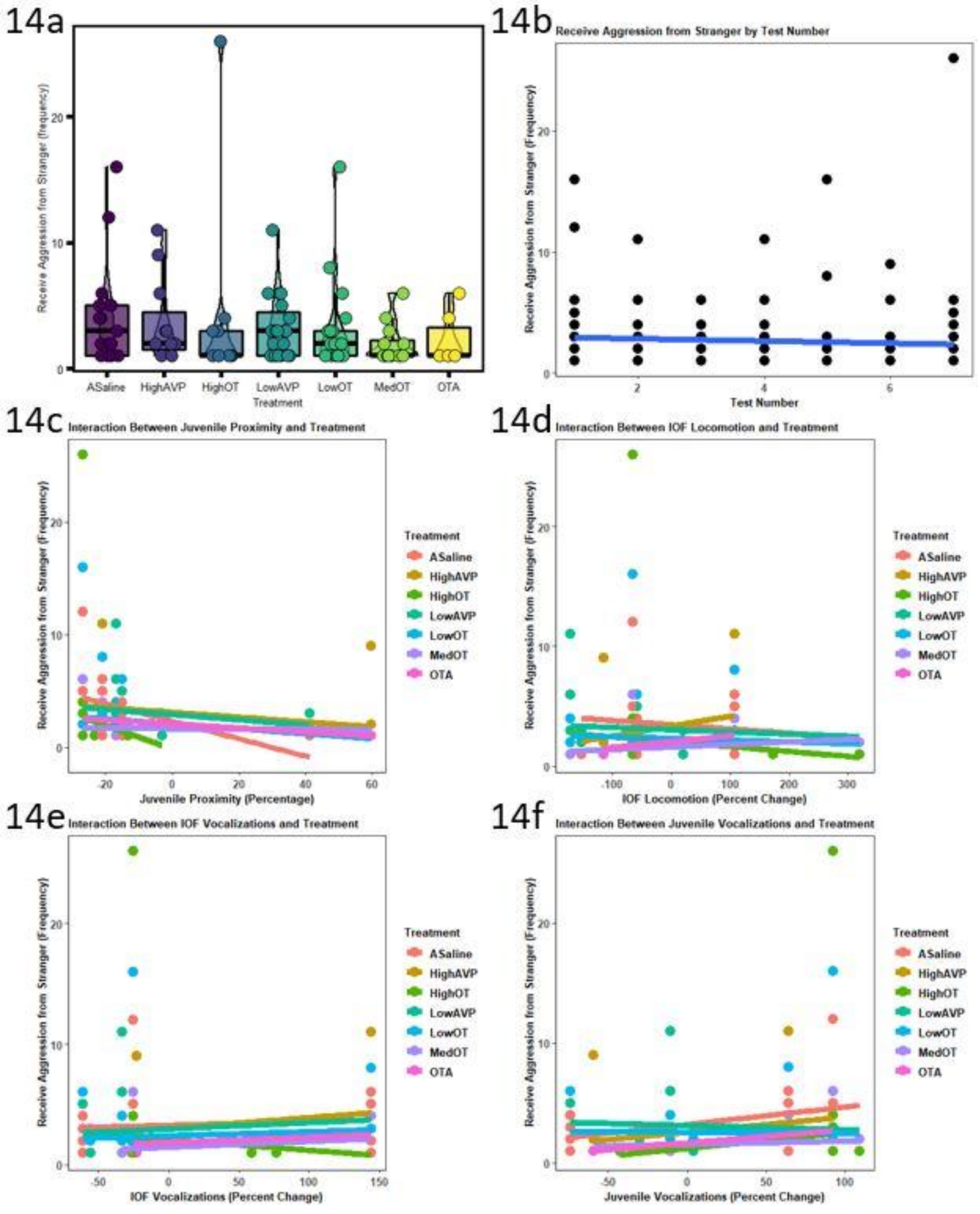


Figure 14. Receive Aggression from Stranger frequency: a) the main effect of *Treatment,* b) *Test Number*, interaction effects between *Treatment* and c) *Juvenile Proximity*, d) *IOF Locomotion*, e)

IOF Vocalizations, and f) *Juvenile Vocalizations.* OT = oxytocin; AVP = arginine-vasopressin; OTA = oxytocin antagonist; ASaline = saline; LowOT = 0.8 IU/kg OT; MeOT = 8.0 IU/kg OT; HighOT = 80.0 IU/kg OT; LowAVP = 40 IU/kg AVP; HighAVP = 80 IU/kg AVP; OTA = 10 mg/kg OTA

SUPPLEMENTARY TABLES

Supplementary Table 1. Log likelihood ratio test results comparing model fit for general linear mixed-effects models and zero-inflated mixed-effects models.

Supplementary 1a. Zone Ratio

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ZoneRatioonlyTreat	9	5356.8	5391.5	-2669.4	5338.8			
ZoneRatioonlySig	23	5311.3	5400.1	-2632.7	5265.3	73.472	14	4.51E-10
ZoneRatioSigInt	27	5309.4	5413.6	-2627.7	5255.4	9.9303	4	0.04162
ZoneRationoObsorTest	51	5316.8	5513.6	-2607.4	5214.8	40.5919	24	0.01846
ZoneRatioAll	53	5316.8	5521.3	-2605.4	5210.8	3.9947	2	0.13569
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	5354.8	5366.3	-2674.4	5348.8			
ZoneRationoObsorTest	51	5316.8	5513.6	-2607.4	5214.8	133.95	48	4.72E-10

Supplementary 1b. Percent Social

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PropSocialonlyTreat	9	3186.9	3221.6	-1584.5	3168.9			
PropSocialonlySig	24	3138.7	3231.3	-1545.3	3090.7	78.2305	15	1.47E-10
PropSocialSigInt	28	3141.8	3249.9	-1542.9	3085.8	4.8368	4	0.3045
PropSocialnoObs	52	3117.8	3318.4	-1506.9	3013.8	72.0051	24	1.08E-06
PropSocialAll	53	3119.7	3324.2	-1506.8	3013.7	0.1289	1	0.7196
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	3185.8	3197.4	-1589.9	3179.8			
PropSocialnoObs	52	3117.8	3318.4	-1506.9	3013.8	165.94	49	1.27E-14

Supplementary 1c. Parent Zone

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PPZonlyPropPar	4	5150.3	5165.7	-2571.2	5142.3			
PPZonlySig	6	5149.5	5172.6	-2568.8	5137.5	4.8014	2	0.09065
PPZnoTreat	7	5146.1	5173.1	-2566	5132.1	5.4138	1	0.01998
PPZonlyTreat	9	5169.9	5204.6	-2576	5151.9	0	2	1
PPZnoObsorTest	37	5130.7	5273.4	-2528.3	5056.7	95.2217	28	2.97E-09
PPZAll	39	5133.7	5284.2	-2527.9	5055.7	0.9616	2	0.61828
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	5162.7	5174.3	-2578.4	5156.7			
PPZnoObsorTest	37	5130.7	5273.4	-2528.3	5056.7	100.06	34	2.00E-08

Supplementary 1d. Stranger Zone

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
SPZonlyTreat	9	2344.4	2379.2	-1163.2	2326.4			
SPZonlySigMain	18	2320.4	2389.9	-1142.2	2284.4	41.989	9	3.30E-06
SPZonlyMain	19	2320.6	2393.9	-1141.3	2282.6	1.8076	1	0.1788
SPZnoJuvReunInt	67	2216	2474.5	-1041	2082	200.6331	48	< 2.2e-16
SPZnoObs	72	2212.2	2490	-1034.1	2068.2	13.7962	5	0.01696
SPZAll	73	2214.2	2495.8	-1034.1	2068.2	0.0001	1	0.99105
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	2361.8	2373.4	-1177.9	2355.8			
SPZnoObs	72	2212.2	2490	-1034.1	2068.2	287.63	69	< 2.2e-16

Supplementary 1e. Other Zone

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ODonlyTreat	9	2465.8	2500.6	-1223.9	2447.8			
ODonlyMain	13	2473.1	2523.2	-1223.5	2447.1	0.7549	4	0.9444
ODonlySigIntandMain	37	2438.3	2581.1	-1182.2	2364.3	82.7692	24	2.19E-08
ODonlySigIntnoTest	42	2408.3	2570.3	-1162.2	2324.3	40.0058	5	1.49E-07
ODonlySigInt	43	2406.7	2572.6	-1160.3	2320.7	3.5936	1	0.058
ODnoObs	72	2389	2666.8	-1122.5	2245	75.6881	29	4.86E-06
ODnoObsorTest	72	2389	2666.8	-1122.5	2245	0	0	
ODAll	73	2390.7	2672.3	-1122.3	2244.7	0.3523	1	0.5528
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	2461.4	2472.9	-1227.7	2455.4			
ODnoObs	72	2389	2666.8	-1122.5	2245	210.35	69	3.29E-16

Supplementary 1f. Parent Grate Touch

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PGTonlyTreat	9	2338.7	2373.4	-1160.4	2320.7			
PGTonlyMain	15	2320.5	2378.3	-1145.2	2290.5	30.2333	6	3.55E-05
PGTonlySigMainandInt	51	2173.5	2370.2	-1035.8	2071.5	218.9819	36	< 2.2e-16
PGTonlySigInt	54	2176.9	2385.2	-1034.4	2068.9	2.613	3	0.45522
PGTnoObsorTest	72	2178.9	2456.7	-1017.5	2034.9	33.9572	18	0.01275
PGTAll	73	2176.7	2458.3	-1015.3	2030.7	4.2806	1	0.03855
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	2340.9	2352.4	-1167.4	2334.9			
PGTAll	73	2176.7	2458.3	-1015.3	2030.7	304.21	70	< 2.2e-16

Supplementary 1g. Stranger Grate Touch

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
SGTonlyTreat	9	1302.4	1337.1	-642.18	1284.4			
SGTonlySigMain	11	1299.9	1342.3	-638.95	1277.9	6.4608	2	0.039541
SGTonlyMain	16	1298.5	1360.2	-633.26	1266.5	11.3807	5	0.044334
SGTnoObs	45	1243.1	1416.7	-576.55	1153.1	113.4153	29	6.28E-12
SGTAll	46	1238.2	1415.7	-573.1	1146.2	6.8988	1	0.008625
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	1316.5	1328	-655.24	1310.5			
SGTAll	46	1238.2	1415.7	-573.1	1146.2	164.27	43	4.52E-16

Supplementary 1h. Zone Change Frequency

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ChangeZoneonlyTreat	9	1835.2	1870	-908.63	1817.2			
ChangeZoneonlyMain	17	1791.5	1857	-878.73	1757.5	59.7887	8	5.13E-10
ChangeZoneSigInt	23	1781.7	1870.4	-867.84	1735.7	21.7921	6	0.00132
ChangeZonenoObs	52	1773.1	1973.8	-834.57	1669.1	66.537	29	8.89E-05
ChangeZoneAll	53	1769.6	1974.1	-831.8	1663.6	5.5376	1	0.01861
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	1850.1	1861.7	-922.06	1844.1			
ChangeZoneAll	53	1769.6	1974.1	-831.8	1663.6	180.52	50	< 2.2e-16

Supplementary 1i. Parent-directed Lip-smacking

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	326.52	346.02	-114.26			
Model9	339.03	348.98	-144.51	60.51	24	1.00E-04
	AIC	BIC	log.Lik	LRT	df	p.value
Model1	326.52	346.02	-114.26			
Model10	410.56	411.76	-202.28	176.04	46	<0.0001

Supplementary 1j. Stranger-directed Lip-smacking

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	969.49	994.56	-421.74			
Model8	1233.38	1246.91	-582.69	321.89	29	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model1	969.49	994.56	-421.74			
Model9	1254.89	1256.88	-622.45	401.4	58	<0.0001

Supplementary 1k. Back-arching/Tail-lashing

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	951.89	976.96	-412.94			
Model2	950.66	974.93	-414.33	2.77	2	0.2502
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	950.66	974.93	-414.33			
Model5	949.31	973.19	-414.66	0.65	1	0.4189
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	949.31	973.19	-414.66			
Model7	1059.4	1076.12	-487.7	146.09	18	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	949.31	973.19	-414.66			
Model8	1479.3	1480.49	-736.65	643.98	57	<0.0001

Supplementary 1l. Stranger Receive Aggression

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	757.69	788.33	-301.85			
Model4	736.75	753.46	-326.37	49.06	35	0.0577
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	736.75	753.46	-326.37			
Model5	738.13	754.45	-328.07	3.39	1	0.0657
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	738.13	754.45	-328.07			
Model6	736.11	752.03	-328.05	0.02	1	0.8746
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	736.11	752.03	-328.05			
Model7	797.88	804.24	-382.94	109.77	24	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	736.11	752.03	-328.05			
Model8	820.9	822.89	-405.45	154.79	35	<0.0001

Supplementary 1a Models:

ZoneRatioonlyTreat: ZoneRatio ~ Treatment + (1 | ID)

ZoneRatioonlySig: ZoneRatio ~ Treatment + PropPar + JuvLoc + PropPar * Treatment +
JuvLoc * Treatment + (1 | ID)

ZoneRatioSigInt: ZoneRatio ~ Treatment + PropPar + InfCarry + IOFProx + IOFGrate +
JuvVoc + JuvLoc + PropPar * Treatment + JuvLoc * Treatment +
(1 | ID)

ZoneRationoObsorTest: ZoneRatio ~ Treatment + PropPar + InfCarry + IOFProx + IOFGrate +
JuvVoc + JuvLoc + PropPar * Treatment + InfCarry * Treatment +
IOFProx * Treatment + IOFGrate * Treatment + JuvVoc * Treatment +
JuvLoc * Treatment + (1 | ID)

ZoneRatioAll: ZoneRatio ~ Treatment + ObsNo + TestNo + PropPar + InfCarry +
IOFProx + IOFGrate + JuvVoc + JuvLoc + PropPar * Treatment +
InfCarry * Treatment + IOFProx * Treatment + IOFGrate * Treatment +
JuvVoc * Treatment + JuvLoc * Treatment + (1 | ID)

Null1: ZoneRatio ~ 1 + (1 | ID)

Supplementary 1b Models:

PropSocialonlyTreat: PropSocial ~ Treatment + (1 | ID)

PropSocialonlySig: PropSocial ~ Treatment + TestNo + PropPar + CnRPar + PropPar *
Treatment + CnRPar * Treatment + (1 | ID)

PropSocialSigInt: PropSocial ~ Treatment + TestNo + PropPar + CnRPar + IOFProx +
IOFGrate + ParAff + IOFVoc + PropPar * Treatment + CnRPar *
Treatment + (1 | ID)

PropSocialnoObs: PropSocial ~ Treatment + TestNo + PropPar + CnRPar + IOFProx +
IOFGrate + ParAff + IOFVoc + PropPar * Treatment + CnRPar *
Treatment + IOFProx * Treatment + IOFGrate * Treatment +
ParAff * Treatment + IOFVoc * Treatment + (1 | ID)

PropSocialAll: PropSocial ~ Treatment + ObsNo + TestNo + PropPar + CnRPar +

IOFProx + IOFGrate + ParAff + IOFVoc + PropPar * Treatment +

CnRPar * Treatment + IOFProx * Treatment + IOFGrate * Treatment +

ParAff * Treatment + IOFVoc * Treatment + (1 | ID)

Null1: PropSocial ~ 1 + (1 | ID)

Supplementary 1c Models:

PPZonlyPropPar: PPZD ~ PropPar + (1 | ID)

PPZonlySig: PPZD ~ JuvReun + PropPar + JuvVoc + (1 | ID)

PPZnoTreat: PPZD ~ JuvReun + PropPar + IOFProx + JuvVoc + (1 | ID)

PPZonlyTreat: PPZD ~ Treatment + (1 | ID)

PPZnoObsorTest: PPZD ~ Treatment + JuvReun + PropPar + IOFProx + JuvVoc + JuvReun *

Treatment + PropPar * Treatment + IOFProx * Treatment + JuvVoc *

Treatment + (1 | ID)

PPZAll: PPZD ~ Treatment + ObsNo + TestNo + JuvReun + PropPar + IOFProx +

JuvVoc + JuvReun * Treatment + PropPar * Treatment + IOFProx *

Treatment + JuvVoc * Treatment + (1 | ID)

Null1: PPZD ~ 1 + (1 | ID)

Supplementary 1d Models:

SPZonlyTreat: sqrtSPZD ~ Treatment + (1 | ID)

SPZonlySigMain: sqrtSPZD ~ Treatment + JuvReun + PropPar + CnRPar + InfCarry +

IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc + (1 | ID)

SPZonlyMain: sqrtSPZD ~ Treatment + TestNo + JuvReun + PropPar + CnRPar +

InfCarry + IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc +

(1 | ID)

SPZnoJuvReunInt: sqrtSPZD ~ Treatment + TestNo + JuvReun + PropPar + CnRPar +

InfCarry + IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc +

PropPar * Treatment + CnRPar * Treatment + InfCarry * Treatment +

IOFProx * Treatment + IOFGrate * Treatment + ParAff * Treatment +

IOFLine * Treatment + IOFVoc * Treatment + (1 | ID)

SPZnoObs: sqrtSPZD ~ Treatment + TestNo + JuvReun + PropPar + CnRPar +

InfCarry + IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc +

JuvReun * Treatment + PropPar * Treatment + CnRPar * Treatment +

InfCarry * Treatment + IOFProx * Treatment + IOFGrate * Treatment +

ParAff * Treatment + IOFLine * Treatment + IOFVoc * Treatment +

(1 | ID)

SPZAll: sqrtSPZD ~ Treatment + ObsNo + TestNo + JuvReun + PropPar + CnRPar +

InfCarry + IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc +

JuvReun * Treatment + PropPar * Treatment + CnRPar * Treatment +

InfCarry * Treatment + IOFProx * Treatment + IOFGrate * Treatment +

ParAff * Treatment + IOFLine * Treatment + IOFVoc * Treatment +

(1 | ID)

Null1: sqrtSPZD ~ 1 + (1 | ID)

Supplementary 1e Models:

ODonlyTreat: sqrtOD ~ Treatment + (1 | ID)

ODonlyMain: sqrtOD ~ Treatment + JuvReun + InfCarry + IOFVoc + JuvVoc + (1 |

ID)

ODonlySigIntandMain: sqrtOD ~ Treatment + JuvReun + InfCarry + IOFVoc + JuvVoc + JuvReun *

Treatment + InfCarry * Treatment + IOFVoc * Treatment + JuvVoc *

Treatment + (1 | ID)

ODonlySigIntnoTest: sqrtOD ~ Treatment + JuvReun + PropPar + InfCarry + IOFProx +

IOFGrate + ParAff + IOFLine + IOFVoc + JuvVoc + JuvReun *

Treatment + InfCarry * Treatment + IOFVoc * Treatment + JuvVoc *

Treatment + (1 | ID)

ODonlySigInt: sqrtOD ~ Treatment + TestNo + JuvReun + PropPar + InfCarry +
IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc + JuvVoc +
JuvReun * Treatment + InfCarry * Treatment + IOFVoc * Treatment +
JuvVoc * Treatment + (1 | ID)

ODnoObs: sqrtOD ~ Treatment + TestNo + JuvReun + PropPar + InfCarry +
IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc + JuvVoc +
JuvReun * Treatment + PropPar * Treatment + InfCarry * Treatment +
IOFProx * Treatment + IOFGrate * Treatment + ParAff * Treatment +
IOFLine * Treatment + IOFVoc * Treatment + JuvVoc * Treatment +
(1 | ID)

ODnoObsorTest: sqrtOD ~ Treatment + JuvReun + PropPar + InfCarry + IOFProx +
IOFGrate + ParAff + IOFLine + IOFVoc + JuvVoc + JuvReun *
Treatment + PropPar * Treatment + InfCarry * Treatment +
IOFProx * Treatment + IOFGrate * Treatment + ParAff * Treatment +
IOFLine * Treatment + IOFVoc * Treatment + JuvVoc * Treatment +
(1 | ID)

ODAll: sqrtOD ~ Treatment + ObsNo + TestNo + JuvReun + PropPar + InfCarry +
IOFProx + IOFGrate + ParAff + IOFLine + IOFVoc + JuvVoc +
JuvReun * Treatment + PropPar * Treatment + InfCarry * Treatment +
IOFProx * Treatment + IOFGrate * Treatment + ParAff * Treatment +
IOFLine * Treatment + IOFVoc * Treatment + JuvVoc * Treatment +
(1 | ID)

Null1: sqrtOD ~ 1 + (1 | ID)

Supplementary 1f Models:

PGTonlyTreat: sqrtPGTD ~ Treatment + (1 | ID)

PGTonlyMain: sqrtPGTD ~ Treatment + JuvReun + CnRPar + InfCarry + IOFProx +
ParAff + JuvVoc + (1 | ID)

PGTonlySigMainandInt: sqrtPGTD ~ Treatment + JuvReun + CnRPar + InfCarry + IOFProx +
ParAff + JuvVoc + JuvReun * Treatment + CnRPar * Treatment +
InfCarry * Treatment + IOFProx * Treatment + ParAff * Treatment +
JuvVoc * Treatment + (1 | ID)

PGTonlySigInt: sqrtPGTD ~ Treatment + JuvReun + PropPar + CnRPar + InfCarry +
IOFProx + IOFGrate + ParAff + IOFLine + JuvVoc + JuvReun *
Treatment + CnRPar * Treatment + InfCarry * Treatment + IOFProx *
Treatment + ParAff * Treatment + JuvVoc * Treatment + (1 |
ID)

PGTnoObsorTest: sqrtPGTD ~ Treatment + JuvReun + PropPar + CnRPar + InfCarry +
IOFProx + IOFGrate + ParAff + IOFLine + JuvVoc + JuvReun *
Treatment + PropPar * Treatment + CnRPar * Treatment + InfCarry *
Treatment + IOFProx * Treatment + IOFGrate * Treatment +
ParAff * Treatment + IOFLine * Treatment + JuvVoc * Treatment +
(1 | ID)

PGTAll: sqrtPGTD ~ Treatment + ObsNo + TestNo + JuvReun + PropPar + CnRPar +
InfCarry + IOFProx + IOFGrate + ParAff + IOFLine + JuvVoc +
JuvReun * Treatment + PropPar * Treatment + CnRPar * Treatment +
InfCarry * Treatment + IOFProx * Treatment + IOFGrate * Treatment +
ParAff * Treatment + IOFLine * Treatment + JuvVoc * Treatment +
(1 | ID)

Null1: sqrtPGTD ~ 1 + (1 | ID)

Supplementary 1g Models:

SGTonlyTreat: logSGTD ~ Treatment + (1 | ID)

SGTonlySigMain: logSGTD ~ Treatment + ObsNo + JuvVoc + (1 | ID)

SGTonlyMain: logSGTD ~ Treatment + ObsNo + TestNo + PropPar + IOFLine + IOFVoc +
JuvVoc + JuvLoc + (1 | ID)

SGTnoObs: logSGTD ~ Treatment + TestNo + PropPar + IOFLine + IOFVoc + JuvVoc +
JuvLoc + PropPar * Treatment + IOFLine * Treatment + IOFVoc *
Treatment + JuvVoc * Treatment + JuvLoc * Treatment + (1 |
ID)

SGTAll: logSGTD ~ Treatment + ObsNo + TestNo + PropPar + IOFLine + IOFVoc +
JuvVoc + JuvLoc + PropPar * Treatment + IOFLine * Treatment +
IOFVoc * Treatment + JuvVoc * Treatment + JuvLoc * Treatment +
(1 | ID)

Null1: logSGTD ~ 1 + (1 | ID)

Supplementary 1h Models:

ChangeZoneonlyTreat: sqrtChangeZone ~ Treatment + (1 | ID)

ChangeZoneonlyMain: sqrtChangeZone ~ Treatment + ObsNo + TestNo + PropPar + CnRPar +
IOFProx + IOFGrate + JuvVoc + JuvLoc + (1 | ID)

ChangeZoneSigInt: sqrtChangeZone ~ Treatment + ObsNo + TestNo + PropPar + CnRPar +
IOFProx + IOFGrate + JuvVoc + JuvLoc + CnRPar * Treatment +
(1 | ID)

ChangeZonenoObs: sqrtChangeZone ~ Treatment + TestNo + PropPar + CnRPar + IOFProx +
IOFGrate + JuvVoc + JuvLoc + PropPar * Treatment + CnRPar *
Treatment + IOFProx * Treatment + IOFGrate * Treatment +
JuvVoc * Treatment + JuvLoc * Treatment + (1 | ID)

ChangeZoneAll: sqrtChangeZone ~ Treatment + ObsNo + TestNo + PropPar + CnRPar +
IOFProx + IOFGrate + JuvVoc + JuvLoc + PropPar * Treatment +
CnRPar * Treatment + IOFProx * Treatment + IOFGrate * Treatment +
JuvVoc * Treatment + JuvLoc * Treatment + (1 | ID)

Null1: sqrtChangeZone ~ 1 + (1 | ID)

Supplementary 1i Models:

```
Model1<-mixed_model(PLS ~ Treatment + ObsNo + TestNo +
 IOFProx + IOFVoc +
 IOFProx*Treatment + IOFVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 IOFProx + IOFVoc +
 IOFProx*Treatment + IOFVoc*Treatment,
 zi_random = ~1|ID,
 iter_EM = 0)
Model9<-mixed_model(PLS ~ Treatment + ObsNo + TestNo +
 IOFProx + IOFVoc +
 IOFProx*Treatment + IOFVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
Model10<-mixed_model(PLS ~ 1,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1j Models:

```
Model1<-mixed_model(SLS ~ Treatment + ObsNo + TestNo +
```

```
 PropPar + IOFProx + JuvLoc +
 PropPar*Treatment + IOFProx*Treatment + JuvLoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + JuvLoc +
 PropPar*Treatment + IOFProx*Treatment + JuvLoc*Treatment,
 zi_random = ~1|ID,
 iter_EM = 0)
Model8<-mixed_model(SLS ~ 1,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + JuvLoc +
 PropPar*Treatment + IOFProx*Treatment + JuvLoc*Treatment,
 zi_random = ~1|ID,
 iter_EM = 0)
Model9<-mixed_model(SLS ~ 1,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
```

Supplementary 1k Models:

```
Model1<-mixed_model(Arch ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(Arch ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 iter_EM = 0)
Model5<-mixed_model(Arch ~ Treatment + ObsNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
```

```
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 iter_EM = 0)
Model7<-mixed_model(Arch ~ Treatment + ObsNo +
 PropPar + IOFProx + IOFVoc,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
 PropPar + IOFProx + IOFVoc +
 PropPar*Treatment + IOFProx*Treatment + IOFVoc*Treatment,
 iter_EM = 0)
Model8<-mixed_model(Arch ~ 1,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1l Models:

```
Model1<-mixed_model(Srecagg ~ Treatment + ObsNo + TestNo +
 JuvReun + IOFLine + IOFVoc + JuvVoc +
 JuvReun*Treatment + IOFLine*Treatment + IOFVoc*Treatment + JuvVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ Treatment + ObsNo + TestNo +
```

```
 JuvReun + IOFLine + IOFVoc + JuvVoc +
 JuvReun*Treatment + IOFLine*Treatment + IOFVoc*Treatment + JuvVoc*Treatment,
 zi_random = ~1|ID,
 iter_EM = 0)
Model4<-mixed_model(Srecagg ~ Treatment + ObsNo + TestNo +
 JuvReun + IOFLine + IOFVoc + JuvVoc +
 JuvReun*Treatment + IOFLine*Treatment + IOFVoc*Treatment + JuvVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ JuvVoc,
 zi_random = ~1|ID,
 iter_EM = 0)
Model5<-mixed_model(Srecagg ~ Treatment + ObsNo + TestNo +
 JuvReun + IOFLine + IOFVoc + JuvVoc +
 JuvReun*Treatment + IOFLine*Treatment + IOFVoc*Treatment + JuvVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
Model6<-mixed_model(Srecagg ~ Treatment + TestNo +
 JuvReun + IOFLine + IOFVoc + JuvVoc +
 JuvReun*Treatment + IOFLine*Treatment + IOFVoc*Treatment + JuvVoc*Treatment,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
```

```
 zi_fixed = ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
Model7<-mixed_model(Srecagg ~ Treatment + TestNo +
 JuvReun + IOFLine + IOFVoc + JuvVoc,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
Model8<-mixed_model(Srecagg ~ 1,
 random = ~ 1|ID,
 data = JPPT1,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
```

CHAPTER 3

Daughters' relationships with their fathers influences proximity maintenance behaviors and glucose uptake during a social separation challenge in adult female titi monkeys

ABSTRACT

Social interactions regulate our behavior and physiology, and strong social bonds can buffer us from stress. Coppery titi monkeys (*Plecturocebus cupreus*) are socially monogamous South American monkeys that display classic attachment behaviors. Infants form selective bonds with their fathers, making them ideal for studying father-daughter bonds. We established a novel method for determining relationship quality in females (n =9). We conducted a preference test between females current and former attachment figures and a neuroimaging study to identify behavioral and neural mechanisms driving female attachment. Across our two experiments, we found evidence to suggest that measures of relationship quality significantly explain variability in behavioral and neural correlates of social bonds in female titi monkeys. In particular, higher-quality relationships with the father established a stronger foundation for forming and maintaining an adult pair bond. We also found evidence to suggest that females are capable of maintaining a relationship with their father at least 6-months post pairing, as evidenced by higher-than-expected levels of proximity maintenance with the father, if they exhibit higher-quality relationships with their fathers. Our examination of neural activity pre- and post-pairing suggest a high degree of overlap between circuitry driving filial attachment and adult pair bonding, as patterns of activity were similar across the two time points, despite overall activity being lower post-pairing ($p < .001$). It is likely that differences in OT, AVP, dopamine, and opioid activity underlie the differences observed in neural activity based on daughter's quality of relationship with their fathers. Findings from the present studies advance current knowledge of

the neurobiological mechanisms foundational to attachment relationships, and help inform how social disruptions caused by events such as the global pandemic may differently impact humans based on the quality of their social bonds.

INTRODUCTION

Social interactions play a regulatory role in behavioral, psychological, and biological processes (Uchino, 2006). For example, couples in more loving relationships, as measured by the Marital Interaction Scale (Braiker & Kelley, 1979), exhibit a positive correlation between relationship experiences and health habits, which leads to perceptions of better overall health (Markey et al., 2007). Strong social support may buffer individuals from stress-induced behavioral decisions and related physiological changes, such as cardiovascular reactivity (Uchino, 2006). The connection between social well-being and physical health may be one factor contributing to greater longevity of individuals who are happy with their social relationships (Diener & Chan, 2011).

For group-living animals, including humans, a lack of social connections and poor social relationships have been associated with adverse health outcomes and increased risk of mortality (Holt-Lunstad et al., 2010). Poor social relationships in particular have been associated with a 29% increased risk for coronary heart disease, and a 32% increased risk for stroke (Valtorta et al., 2016). Understanding mechanisms underlying social bonds is especially critical now because the COVID-19 pandemic has detrimentally impacted human social relationships (Evans et al., 2020; Luetke et al., 2020), which may result in adverse health outcomes (Saggioro et al., 2021). Women may be particularly susceptible to psychiatric disorders of social behavior and, compared to men, are more likely to be diagnosed with affective disorders such as major depressive disorder (Weissman & Olfson, 1995). It is possible that social risk factors may

contribute to women's increased likelihood of developing affective disorders (Kuehner, 2017). It is therefore important to study how social bonds may affect health outcomes, particularly in women.

A social bond is defined as a close, selective, enduring, interpersonal relationship that is maintained by physiological and behavioral mechanisms (Bales et al., 2021; Lim & Young, 2006). Bowlby (Bowlby, 1977) argued that humans display attachment behaviors (e.g., seeking proximity with preferred individuals, distress upon involuntary separation from attachment figures) throughout their lives, and Ainsworth (Ainsworth et al., 1978) examined social bonds through the lens of attachment theory. Bonded individuals often display physiological and behavioral synchrony, and the degree of this coordination has previously been shown to be predictive of bonding strength in humans (Feldman, 2007, 2012b, 2012a). Various types of social bonds exist across species, including mother-offspring bonds, pair bonds, and friendships.

A monogamous pair bond is an enduring, selective bond between two adults (Bales et al., 2021), exemplified by a preference for close physical proximity to a specific individual (Carp et al., 2016), aggression towards unfamiliar intruders (Winslow et al., 1993), distress upon separation from the pair bonded individual (Mendoza & Mason, 1986a), and the ability of partners to buffer each other from stressors (S. Cohen & Wills, 1985; Heinrichs et al., 2003). Mating also plays a large role in facilitating and maintaining strong affiliative bonds between adults (Lim & Young, 2006; Numan, 2015), but is not necessary for a pair bond (Bales et al., 2021).

Titi monkeys (*Plecturocebus spp.*) are socially monogamous South American monkeys that live in the Amazon basin (Brazil, Bolivia, Peru, Ecuador, and Colombia). In the wild, titi monkeys live in small family groups and raise their offspring together (Fuentes, 1998). Titi

monkeys also display classic attachment behaviors (Fuentes, 1998). For example, titi monkeys maintain proximity with their partners in the lab (Karaskiewicz et al., 2021; Rothwell et al., 2020) and in the wild (Dolotovskaya & Heymann, 2020; Dolotovskaya, Walker, et al., 2020; Kinzey & Wright, 1982), potentially as a means of maintaining pair bonds. Titi monkeys also exhibit distress upon separation from their attachment figures and stress buffering by their attachment figures (Hoffman, 1996; Hoffman et al., 1995; Mendoza & Mason, 1986a; Valeggia, 1996). Because titi monkeys exhibit attachments and family structures similar to humans, they are an excellent model species for research topics with human health applications.

Interestingly, the primary attachment figure for titi monkey infants is the father. The fathers spend more time carrying their infants, and when infants are given a choice between interacting with their mothers or fathers, they will choose their fathers (Mendoza & Mason, 1986b). It is likely that infants form a strong attachment to their fathers because fathers spend more time carrying their infants than mothers (Mendoza & Mason, 1986b). Mothers only tolerate holding infants for feeding, and females have been observed immediately soliciting for the male to take the infant once feeding is done and actively avoiding the infant when not nursing in the wild (Dolotovskaya & Heymann, 2020) and in captivity (Karaskiewicz et al., 2021).

Additional evidence for a selective attachment to the father comes from studies on behavioral and physiological responses to separation from and reunion with the father. Titi monkey infants will show behavioral signs of distress upon separation from their fathers, but virtually no response when separated from their mothers if the father is present (Hoffman et al., 1995). For example, infants separated from their attachment figures will show increased vocalizations and locomotion (Mendoza & Mason, 1986b), behaviors which serve as measures of anxiety in NHPs (Barros & Tomaz, 2002; Hennessy et al., 1995). Infants also exhibit a

significant rise in plasma cortisol (CORT) levels when separated from the father, and only reunion with the father will attenuate that rise in CORT (Mendoza & Mason, 1986b). Anxiety-like behaviors are also attenuated in the presence of the father, but not the mother.

This selective attachment to the father persists as titi monkeys mature. Even at 27 months of age, female titi monkeys exhibit a sustained elevation in CORT throughout a 5-day separation period, and CORT levels return to baseline only upon reunion with parents (Valeggia, 1996). Daughters as old as 3.5 years of age that are reintroduced to natal groups following one month of social separation are able to rejoin their group and similarly show a reduction in hypothalamic-pituitary-adrenal (HPA)-activity following reunion (Hoffman, 1996). Taken together, these findings suggest that the filial bonds between daughters and their father are long-lasting and continue past puberty (previously estimated to occur between 13-15 months [Valeggia, 1996] but more recently estimated to occur closer to 30 months [Conley et al., 2022] for female titi monkeys).

When titi monkeys are paired with an unfamiliar opposite-sex conspecific as adults, they form an exclusive emotional attachment similar to the filial bond they previously exhibited with their fathers (Cubicciotti, 1976). In fact, pair-mates become each other's exclusive attachment figures (Mendoza & Mason, 1986b). When separated from a pair-mate, titi monkeys show prolonged, elevated levels of circulating CORT; however, separation from an infant or sibling does not result in the same heightened CORT response (Mendoza et al., 2000; Mendoza & Mason, 1986b). CORT levels only return to normal when a titi monkey is reunited with his/her partner, and reunion with a non-attachment figure does not attenuate their stress response.

While we know that as juveniles the father is the primary attachment figure and as adults the primary attachment figure is the partner (Mendoza & Mason, 1986b, 1986a), a gap in

knowledge exists with regards to the transfer of primary attachment from the father to the partner in titi monkeys. And while titi monkeys consistently exhibit strong, selective attachments, there is a large degree of variability in the degree to which they display behavioral correlates of attachment, such as proximity maintenance, both in the lab (Carp et al., 2016; Hoffman, 1996; Mayeaux et al., 2002) and in the wild (Dolotovskaya, Walker, et al., 2020). Titi monkey interactions are particularly variable and unpredictable at the beginning of a new relationship (Hoffman, 1996; Tardif et al., 2006). It is possible that variability in infant-father attachment relationships may help explain some of this variability in adult attachment behaviors.

Attachment relationships in adolescence have been found to influence adult attachments in humans (Dinero et al., 2008). For humans, relationships with parents may be especially influential at the beginning of a new romantic relationship (Dinero et al., 2022). As the individual gets older and is involved in more romantic relationships, the influence of their relationship with their parents weakens, and future relationships are more heavily influenced by previous romantic relationships. Therefore, it is especially important to investigate that first transition to adult romantic attachment if we want to understand the influence of filial attachment on adult relationships in titi monkeys.

In the wild, emigration from the natal group may be driven by both a decrease in affiliation with family members and an increase in interest in unfamiliar conspecifics (Mayeaux et al., 2002). By 18 and 24 months of age, females in particular appear more willing to interact with unfamiliar conspecifics, particularly unfamiliar pairs of adult titi monkeys (Mayeaux, Mason & Mendoza, 2002). In the wild, around 3 to 4 years of age, titi monkeys naturally emigrate from their family groups without any obvious social pressure from parents or siblings

(Bossuyt, 2002; Norconk, 2011; Souza-Alves et al., 2019; Van Belle et al., 2016), with females in particular seeming to disperse sooner than males (Bossuyt, 2002).

It is unclear how father-daughter relationships influence this transition from living in the natal group to finding a partner and forming a long-lasting pair bond with an opposite-sex conspecific. Even if females do not emigrate very far from their natal territory (Hoffman, 1996), they should eventually prefer to spend more time in proximity with their partner than their fathers. The timeline for this change in preference, however, is unknown. It is likely that the daughter's relationship with her father may impact how she interacts with a new partner. In human studies, parents' relationship quality has been found to be a predictor of children's future romantic relationship quality (Gager et al., 2016); therefore, a measure of relationship quality may help us better understand variation in titi monkey pair bonds. In fact, one study has demonstrated an effect of infant attachment on adult relationships in titi monkeys (Savidge & Bales, 2020). In particular, subjects that were more distressed upon separation from their father during an infant social challenge were more affiliative with their partners during the first six months of their first pairing. Subjects that were better buffered from stress by their fathers, as evidenced by decreased anxiety-like behaviors during an infant social challenge, also exhibited lower anxiety-like behaviors in the presence of their partners during a novelty response paradigm (Savidge & Bales, 2020). It is unknown, however, *how* variation in infant and adolescent relationship quality influences later attachment behaviors. It is likely that neurobiological mechanisms important for social bonding and stress buffering underly this variability in attachment behaviors.

The majority of our understanding of the neural correlates of pair bonding comes from research using prairie voles (Bales et al., 2017). Most published neuroimaging studies conducted

on titi monkeys have only used males as the subjects (Bales et al., 2017); however, some recent publications have examined glucose uptake in males and females (Arias del Razo et al., 2020, 2022). These studies have helped pinpoint regions of interest for understanding the neurobiology of monogamy. Brain areas important for reward circuitry (ventral pallidum [VP], nucleus accumbens [NAc]), social recognition circuits (lateral septum [LS], medial amygdala [MeA], medial preoptic area [MPOA]), and areas where oxytocin (OT) and arginine-vasopressin (AVP) are produced (supraoptic nucleus of the hypothalamus [SON]) have been found to be involved in titi monkey pair bonding (Arias del Razo et al., 2022; Bales et al., 2007).

OT activity in the NAc and AVP activity in the VP are important for the facilitation of persistent, selective attraction found in pair bonded prairie voles. OT in the amygdala and AVP in the LS are thought to be important for social recognition (Lim & Young, 2006), which is necessary for the formation of a persistent, selective attachment. OT and AVP also act in conjunction with dopamine (DA) to facilitate the rewarding aspects of pair bonding (Lim & Young, 2006; K. A. Young et al., 2010). Specifically, activation of DA-type 2 receptors (D2) and OT receptors (OTR) in the NAc occurs during partner preference formation. The NAc projects to the VP, where AVP also acts to facilitate the pair bond. After a pair bond is formed, DA-type 1 receptors (D1) are upregulated in the NAc in prairie voles, and this upregulation is thought to increase agonistic behaviors towards unfamiliar conspecifics, maintaining the pair bond (K. A. Young et al., 2010).

In titi monkeys, short-term separations (48 hours) between partners resulted in decreased glucose uptake in the VP, LS, paraventricular nucleus of the hypothalamus (PVN), periaqueductal gray (PAG), and cerebellum, along with an increase in CSF OT, and plasma CORT and insulin (Hinde et al., 2016). It is hypothesized that these changes may be due to the

relations between the HPA-axis, which can exert negative feedback on the PVN, and interactions between the LS, NAc and VP. In particular, the LS normally inhibits the NAc, which inhibits the VP; therefore, when LS activity is decreased, the inhibition of the NAc is lower, which increases the inhibition of the VP. Finally, OT rises in response to relationship distress, which influences changes on the PAG and cerebellum (Hinde et al., 2016).

Given that we see considerable behavioral overlap between daughter's attachment to their fathers and partners' attachment to each other, it seems likely that the neurobiology of filial attachment and adult pair bonds is similar. Indeed, it is thought that the neural circuitry underlying parental care and monogamy are close approximations of each other (Numan, 2015), and it is thought that the ability to display monogamy and paternal care evolved somewhat close together (Lukas & Clutton-Brock, 2013). One hypothesis is that adult pair bonds may have evolved from the physiological mechanisms mediating parent-offspring bonds (Carter, 1998; Fernandez-Duque, Valeggia, & Mendoza, 2009), but modified to include additional systems that promote reproductive behaviors (Van Anders, Goldey, & Kuo, 2011).

One brain region that may differ between infants' attachment to parents and partner's attachment to each other is the amygdala. A neuroimaging study on human maternal and romantic love show a deactivation of the amygdala, which may serve to suppress neural circuitry associated with social assessment and negative emotions, possibly reducing avoidance of others (Bartels & Zeki, 2004). Bilateral lesioning of the amygdala in rhesus macaques increases social affiliation and reduces inter-animal distances (Emery et al., 2001), which seems to support the idea that suppression of amygdala activity may reduce social avoidance and allow for increased affiliation. Therefore, inhibition of the amygdala may play a role in facilitating interactions between unfamiliar conspecifics, allowing for the initial formation of a pair bond with a specific

individual. In general, the amygdala does not seem to be as involved in infant attachment as it is for adult romantic attachment. A lesion study using rhesus macaques found that lesioning of the amygdala at two weeks of age did not affect infants' bonds with their mothers, but rather reduced social inhibition at six months of age (Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004). Studies in rodents and humans also suggest the amygdala does not play a role in infant attachment (for a review, see Sullivan, Perry, Sloan, Kleinhaus, & Burtchen, 2011).

Our understanding of the neural correlates of infant attachment, and how it compares to adult romantic attachment within an individual is severely lacking. Currently, the majority of our understanding of attachment in titi monkeys comes from adult males. Therefore, it is particularly important to identify brain regions that are similarly activated in parallel paradigms when titi monkey females are tested with their fathers and with their partners. It is likely that the quality of a daughter's relationship with her father may influence neural activity; therefore, it is essential to investigate how variability in relationship quality impacts activity in brain regions associated with social bonding.

Our main objectives were to understand how relationship quality impacts the behavioral correlates of proximity maintenance and the neural correlates of separation distress and stress buffering. To better understand the role of relationship quality on female attachment behavior and neural activity, we completed two separate pre- and post-pairing experiments: 1) a preference test between the father and the partner and 2) a neuroimaging study. We had several specific predictions for our two experiments:

Experiment 1 Predictions

1. During the first preference test (1-week pre-pairing), we expected females to prefer spending more time in proximity to their father than the unfamiliar male.

2. Preference for the father was predicted to still be higher than preference for the partner during the 1-week post-pairing preference test, based on findings from titi monkey research indicating attachment persist following a 1-week separation period (Fernandez-Duque, Mason, & Mendoza, 1997). However, by 6-months post-pairing we expected females to show a preference for their partner over their father, as this is the point when females have formed a strong bond with their partner (Rothwell et al., 2020).
3. We predicted that indicators of a higher-quality relationship with the father would be positively correlated with the time spent in the father's preference zone, particularly during the first two preference tests (1-week pre-pairing and 1-week post-pairing).
4. We also predicted females that exhibit a higher-quality relationship with their father would have a stronger foundation for forming a strong adult pair bond and would therefore exhibit a stronger preference for their partner 6-months post-pairing.

Experiment 2 Predictions

1. Given that male titi monkeys exhibited an increase in whole-brain glucose uptake post-pairing in previous neuroimaging studies (Bales et al., 2007; Hinde et al., 2016), we expected to see increases in whole brain glucose uptake as well as uptake in the social salience network (LS, NAc, VP, Amygdala, Hypothalamus, VTA), PAG, and cerebellum when females were tested 6-months post-pairing relative to when they are tested while still in their natal group.

2. While the NAc and hypothalamus did not show significant changes in male titi monkeys in the separation study by Hinde and colleagues (Hinde et al., 2016), we predicted that we would see decreased activity in these brain regions in females in response to separation from their attachment figure (Figure 1). We predicted this because OT is produced in the SON, and OT activity in the NAc is found to be particularly important in female pair bonding in rodents (K. A. Young et al., 2010).
3. The VTA also has important projections to the NAc (Haber & Knutson, 2009), and we predicted it may play a role in females' response to separation from her attachment figure, even though it does not seem to play a role in males (Hinde et al., 2016). In particular, we predicted lower activation of the VTA when females are separated from their attachment figure compared to when they are tested with their attachment figure (Figure 1).
4. We predicted amygdala activity would not significantly differ between the baseline and separation conditions for females while in the natal group, as the amygdala has not been shown to be activated in studies of infant attachment in other animal models (Sullivan et al., 2011). However, we predicted we would see decreases in glucose uptake in the amygdala when females are separated from their partners 6-months post-pairing (Figure 1).

METHODS

Subjects & Housing

Subjects were nine female titi monkeys (ages 27-36 months), their fathers, and their partners (N = 9 females; N = 9 fathers; N = 9 partners). This age range represents a time when

females are likely to emigrate from their natal group in the wild and form a pair bond (Bossuyt, 2002; Souza-Alves et al., 2019; Thompson & Norconk, 2011; Van Belle et al., 2016). All females were paired with vasectomized males so they would not reproduce during this study. Mates were selected based on genetic relatedness and availability to be paired. For example, males had to have entered puberty, which is estimated to be around 22 months of age for males (Conley et al., 2022). Males also could not be in a current pair bonded relationship so they would be available to form a bond with our subjects. Even though females were not able to get pregnant during testing, we still collected urine samples three times per week to track subjects' reproductive status.

Subjects were housed at the California National Primate Research Center. They lived in their natal groups with their parents and any older siblings that were still in the natal group prior to 29 months of age. Then females lived with their partner once they were paired around 29 months. Families were housed in a 1.2 m x 1.2 m x 2.1 m stainless steel cage or a 1.2 m x 1.2 m x 1.8 m with four horizontal perches, a food bowl, and two water dispensers. They were fed twice daily with Purina New World monkey chow, rice cereal, carrots, apples, and bananas. They were kept on a 12 hour light, 12 hour dark cycle, with lights coming on at 0600 hours and turning off at 1800 hours. The room temperature was maintained at approximately 21°C. For additional information regarding animal husbandry, please see Tardif and colleagues (Tardif et al., 2006) and Mendoza and Mason (Mendoza & Mason, 1986a). This study was approved by the IACUC of the University of California, Davis; and complied with legal requirements of the United States.

To study how relationship quality impacts the behavioral correlates of proximity maintenance and the neural correlates of separation distress and stress buffering, we conducted two experiments simultaneously (Figure 2):

Experiment 1 Data Collection

Females were tested in a total of three preference. Subjects were released into the center chamber of the testing apparatus (see Figure 3 for testing apparatus) and allowed to freely move about the center arena for the duration of the test. Briefly, tests lasted for approximately 3 hours, and we live-scored five consecutive 30-minute blocks. The first preference test occurred when the female was still in her natal group. Her father was on one side, and an unfamiliar male (with whom she was later paired) was on the other side (see Figure 3 for testing apparatus). Testing was then repeated 1-week post-pairing and 6-months post-pairing (Figure 2). The side that stimulus animals were on alternated between tests to avoid development of a side preference. Subjects and stimulus animals remained in their respective area of the testing apparatus for the duration of the test. For a detailed description of preference testing procedures, see Carp and colleagues (Carp et al., 2016).

All tests were video-recorded and live-scored using Behavior Tracker (www.behaviortracker.com) using an established ethogram (Table 1). We quantified the amount of time a subject spent in the preference zone of their partner and their father. We also scored measures of affiliation (e.g., lip-smacking, long calling), arousal (e.g., back-arching/tail-lashing, chest rubbing), aggression (e.g., exhibiting and receiving aggression), and anxiety (e.g., changing preference zones). For affiliative and aggressive behaviors, we also recorded which stimulus animal the behavior was directed towards. We chose to measure these additional behaviors to get a better understanding of how measures of daughter's relationship quality with her father may be

affecting affiliative and agonistic interactions with stimulus pairs as well as how they affect arousal and stress responses of the subject. We also recorded vocalizations using a Marantz PMD 660 solid-state digital flash recorder and a RODE NTG-2 directional condenser microphone. At the end of the five observations, we completed five catch and release sessions. We caught the subject, released her into the center arena, and if she stayed in one preference zone for 10 consecutive seconds that was scored as her choice. If she did not stay in either preference zone for 10 consecutive seconds after five minutes, we designate that observation as "no choice". After catch and release, all titi monkeys were returned to their respective home-cage.

Experiment 2 Data Collection

In tandem with Experiment 1, females were tested in a total of four positron emission tomography (PET) scans: two while she was in her natal group and her primary attachment figure was her father, and two 6-months post pairing, when females demonstrate a clear preference for their partner over strangers (Rothwell et al., 2020). All PET scans were counter-balanced (Figure 1). At the completion of all four PET scans, we conducted one structural magnetic resonance imaging (MRI) scan to use for co-registration and quantification of [18F]-fluorodeoxyglucose (FDG; PETNET Solutions, Sacramento, CA, USA) uptake. FDG uptake has previously been used in titi monkeys as an approximation of brain activity (Arias del Razo et al., 2020, 2022; Bales et al., 2007; Hinde et al., 2016).

The first two PET scans occurred when the female was still in her natal group. We examined glucose metabolism when the female was scanned with her father, and when she was separated from her father for 30 minutes. The scan conducted while our subject is with her father represented the "baseline" scan, while the scan conducted while she was alone allowed us to measure neural correlates of distress upon separation from her primary attachment figure. Our

methods were similar to those used in the study by Hinde and colleagues (Hinde et al., 2016); however, instead of a 48-hour separation, we separated females from their fathers for 30 minutes.

Females and their families were relocated to the testing room 48-hours prior to the start of the scan to reduce the effects of being in a novel environment on neural activity (Hinde et al., 2016). Titi monkeys were fasted for 10 hours prior to the start of each PET scan, with water available ad libitum. On the day of the PET scan, females were hand-caught and received a bolus FDG injection into the saphenous vein. The father remained in the testing cage during the "baseline" condition, or was removed from the room while the female received her FDG injection during the "separation" condition. The mother and any siblings were removed from the testing room in both conditions when the female received her FDG injection. The female was returned to the testing cage (where she was either alone or with her father), and filmed for 30 minutes during the conscious uptake period.

Following the 30-minute uptake period, the females were hand-caught and sedated with ketamine (25 mg/kg IM). We collected a 1.0 ml blood sample via femoral venipuncture. Following blood collection, samples were placed on ice immediately, centrifuged at 1,610 x *g* at 4ºC, and the plasma extracted and stored at -80ºC until assay.

PET imaging was performed on a microPET P4 scanner (Siemens Preclinical Solutions, Knoxville, TN, USA). Anesthesia was maintained throughout the scan with isoflurane (1-2%). Scans were acquired for 60 minutes. At the completion of the study, MRI scans were conducted in a GE Signa LX 9.1 scanner (General Electric Corporation, Milwaukee, WI, USA) with a 1.5 T field strength and a 3" surface coil. For further details regarding PET and MRI methods, see Hinde and colleagues (Hinde et al., 2016) and Arias del Razo and colleagues (Arias del Razo et al., 2020, 2022) .

Region of interest (ROI) structures were drawn on each subject's MRI image using PMOD (version 4.2) software (https://www.pmod.com/web/) using the "view" tool. The ROIs for this study were the following: VP, NAc, VTA, LS, Amygdala, Hypothalamus, PAG, and Cerebellum. Positions of ROIs were consistently estimated across subjects using clearly visible landmarks (Supplementary Figure 1). The thickness of slices were 1mm, so some ROIs were only on one slice (LS, VTA, VP), while others were on two (NAc, PAG), three (Amygdala, Hypothalamus), or many (cerebellum) slices. PET scan data were then co-registered with the same MRI image for each subject using the "fusion" tool in PMOD. To analyze ROI activity, we extracted the total activity for each ROI (left and right), which was calculated as Standardized Uptake Value by body weight (SUVbw).

Females were then paired and remained with this partner for the duration of testing. Following 6-months of pairing, females were tested using the same paradigm as described above; however, they were tested with their partner or alone (separation condition). This second set of scans allowed us to compare glucose uptake in our same ROIs when the same female was temporarily separated from her partner, with whom she has formed a pair bond.

Quantification of Relationship Quality

We have access to several measures of infant and juvenile relationship quality in our captive titi monkey colony. For the present study, we quantified several indicators of separation distress and proximity maintenance for our 9 subjects (Table 2). Measures were collected both as scan samples and as responses to social challenges when females were infants and juveniles. For data analysis, all measures of relationship quality were centered about the mean value for our 9 subjects. This allowed us to determine how variation in relationship quality influences various outcomes in our testing paradigm. For example, we could examine how females that spend a

greater proportion of time in proximity to their fathers as infants differ from females that spend comparatively less time maintaining proximity with their fathers. Below are details regarding data collection for each measure of relationship quality:

IOF Locomotion

All infant titi monkeys at the California National Primate Research Center undergo infant open field (IOF) tests when they are four months of age. This test measures responses to a novel environment and reactions to the presence of family members during a stressful experience. Subjects are placed in the center of a one meter by one meter arena with a six-by-six square floor grid. One wall included a wire mesh grate that allowed auditory, visual, and olfactory access to a stimulus animal that was placed in a transport box on the other side of the grate. During this testing, subjects experienced four five-minute trials, each with a different stimulus on the other side of the grate (their father, their mother, their oldest sibling [if they had any], and an empty box). For a more detailed description of the infant open field methods, see Larke and colleagues (Larke et al., 2017) and Savidge and Bales (Savidge & Bales, 2020). During testing, we count the number of lines that the infant crosses within each five-minute trial (defined as two or more limbs crossing a line; Larke et al., 2017). For the present study, we calculated the number of line crosses for each subject during the empty box condition and the father condition. We then calculated the percent change in line crosses from the father condition to the empty box condition ([{line crossing when alone – line crossing with father} / line crossing with father] * 100). We interpreted a greater increase in line crossing to indicate heightened distress upon separation, which may be an indicator of a higher-quality relationship with the father.

IOF Vocalization

During IOF testing we also score infant vocalizations. During this test we typically only hear infants make trill calls (as defined by Clink et al., 2019), but if they made any other separation distress calls, we combined those with the number of trills scored to create a total vocalization score. We then calculated the percent change in vocalizations from the father condition to the empty box condition. A greater increase in vocalizations was interpreted as an enhanced separation distress response, which is assumed to be an additional indicator of a higher-quality relationship with the father.

Juvenile Locomotion

We quantified measures of juvenile relationship quality using behaviors scored during the saline condition of our social separation testing described in Chapter One. We quantified the duration of time females spent locomoting when females were tested alone (separation distress condition) and when they were tested with their father (stress buffering conditions), and calculated the percent change in locomotion from the stress buffering to the separation distress condition. We interpreted a greater increase in locomotion as a heightened separation distress response, which may be indicative of a higher-quality relationship with the father.

Juvenile Vocalization

We also quantified percent change in separation distress vocalization frequency from the stress buffering to the separation distress conditions of the social separation test described in Chapter One when females were treated with saline. The values for this measure were much larger than the values for all other relationship quality variables; therefore, we rescaled this measure by dividing by 10 to make this variable on a more similar scale to our other predictors. A greater increase in vocalizations from the stress buffered to the separation distress condition

represented an elevated stress response, which we interpreted as a higher-quality relationship with the father.

Juvenile Proximity

We also quantified measures of juvenile proximity maintenance using video recordings from our social separation testing described in Chapter One. Females were reunited with their families in their home-cage immediately following blood sample collection, and their interactions were recorded for 15 minutes. For this measure, we only looked at behaviors during the reunion period following the saline condition of the separation distress condition (when females were left alone in the home-cage for 30 minutes). We calculated the duration of time that females spent in proximity, contact, or tail-twining with their fathers during this 15-minute reunion period (for detailed definitions of these behaviors, see Karaskiewicz et al., 2021)). We calculated the percentage of time females were in social proximity with their fathers as the total time in social proximity/contact/tail-twining out of the entire 15-minute reunion period ([duration of time in contact/proximity/tail-twining / 600 seconds] * 100). We interpreted a greater percentage of time maintaining social proximity to the father as a higher-quality relationship with the father.

Juvenile Parent Preference

We quantified additional measures of juvenile proximity maintenance using data from the saline condition of parent preference testing described in Chapter Two. We calculated the percentage of time females spent in the preference zone of their parents during the entire 3-hour parent preference test by dividing the total time in the parent zone by 150 minutes. We interpreted a greater percentage of time in the parents' preference zone as an indication of a higher-quality relationship with the father.

Juvenile Parent Choice

We also used data from the catch and release sessions described in Chapter Two when females were treated with saline. We calculated the percentage of times females chose their parents during the five catch and release bouts by dividing the number of times the female chose her parents by five. We assumed females that chose their parents more often had a higher-quality relationship with their father.

IOF Proximity

In addition to quantifying measures of separation distress during IOF testing, we were also able to quantify measures of infant proximity maintenance. We used the same definition of grate proximity that Larke and colleagues (Larke et al., 2017) used to quantify percent time infants spent in close proximity to the grate in the IOF arena. We calculated the percentage of time subjects spent in proximity to the grate during the father condition by dividing the amount of time in proximity to the father by 300 seconds. We assumed greater increase in percentage of time spent in close proximity to the father during testing indicated a higher-quality relationship with the father.

IOF Grate

We also quantified the amount of time the females spent physically touching the grate in the IOF arena. Females had to have at least one hand or foot on the grate to be considered touching the grate (touching the grate with the tail or a different part of the body did not count as grate touching in this test to mirror how grate touching is scored in preference tests [Carp et al., 2016]). We calculated the percentage of time spent touching the grate during the father condition of IOF testing as an additional measure of infant proximity maintenance. We also interpreted a

greater percentage of time spent touching the grate as indicating a higher-quality relationship with the father.

Infant Proximity

From the day an infant is born until they are nine months old, we conduct spot checks every two hours, five days per week, to record who is carrying the infant (mother, father, or sibling) or whether the infant is out of contact with any family member and independently moving about the home-cage (Karaskiewicz et al., 2021). The percentage of time spent being carried by a family member out of all checks recorded can give us a measure of the quality of parental care an infant receives. For the present study, we quantified the percentage of time females were carried by their father out of all scan samples collected for that subject. We assume greater percentage of time being carried by the father early in life could be a predictor of a better father-daughter relationship in this species, and possibly result in a higher-quality relationship with the father.

Pair Affiliation

In addition to the infant scan samples, as soon as a titi monkey pair is established, our lab conducts pair-mate scan samples every two hours to record where pair-mates are in relation to each other (Karaskiewicz et al., 2021). The four behaviors recorded on our check sheets are proximity, contact, tail-twining, and nothing. Check sheets are completed five days per week for the entire time that a pair is together. This measure of pair-mate affiliation gives us an indication of the overall level of affiliation between partners. We assume greater percentage of time in affiliative contact is an indicator of higher quality relationships in this species. For the present study, we quantified the percentage of time partners were in proximity, contact, or tail-twining out of all scan samples collected on the parents during the first 6 months of pairing.

Parent Affiliation

We also quantified affiliation for the parents of our subjects when they were infants and juveniles using the same scan samples used to measure *Pair Affiliation*. This measure of pair-mate affiliation gives us an indication of the overall level of affiliation that the infant observes in the natal group. In human studies, parents' relationship quality has been found to be a predictor of children's future romantic relationship quality (Gager et al., 2016); therefore, this measure may help us better understand variation in titi monkey attachment. We assume greater percentage of time in affiliative contact is an indicator of higher quality relationships in this species, and may set the foundation for infants to form higher-quality relationships. For the present study, we quantified the percentage of time parents were in proximity, contact, or tail-twining out of all scan samples collected on the parents during the first 14 months of our subjects' lives.

Data Analysis Experiment 1

All analyses were conducted in R Statistical Software (version 4.0.3, R Core Development Team, 2020). For all outcome measures, we performed a Shapiro Wilk test of normality and transformed non-normally distributed variables as necessary (Royston, 1983). All tests were two-tailed and the significance threshold was set at .05.

We first identified which of the 12 relationship quality variables (*IOF Locomotion, IOF Vocalization, Juvenile Vocalization, Juvenile Locomotion, Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, Infant Proximity, IOF Proximity, IOF Grate, Pair Affiliation, Parent Affiliation;* Table 3) best explained variance in our outcome variables (Table 1). To identify best-fitting relationship quality variables, we ran stepwise regression using the leaps package (Lumley, 2020). This method allowed us to iteratively add and remove variables in the predictive model to identify which subset of variables resulted in the model with the

lowest prediction error (Bruce & Bruce, 2017; Gareth et al., 2014). To simplify the stepwise regression models, we first ran separate stepwise regression models for separation distress (*IOF Locomotion, IOF Vocalization, Juvenile Locomotion, Juvenile Vocalization*), proximity maintenance (*Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, IOF Proximity, IOF Grate, Infant Proximity*) and affiliation (*Pair Affiliation, Parent Affiliation*) variables. Once we had identified the top separation distress, proximity maintenance, and affiliation variables, we ran a final stepwise regression model that just included those top variables from each category (separation distress, proximity maintenance, affiliation). The combination of relationship quality variables that was identified as producing a model with the lowest prediction error was then used in our mixed-effects models.

For Zone Ratio, Percent Social, Parent Zone, Stranger Zone, Other Zone, Parent Grate Touch, Stranger Grate Touch, and Zone Change Frequency, we ran general linear mixed-effects models (LMM) using the lmerTest package (Kuznetsova et al., 2017), with animal identity as a random effect to account for repeated measures. Our models were specified as: $y_{ij} = \beta_0 + \beta_1 x^{(1)}_{ij} + \cdots + \beta_m x^{(m)}_{ij} + \eta_{i0} + e_{ij}$. In this model, y_{ij} denotes the *j*th observation taken of individual *i* for outcome variable *y.* Individual covariates included in the model are represented by *x* (covariates 1-m), which have fixed effects common to all individuals in the population (β). This model also includes a random effect of ID that is specific to each individual (η_{i0}). Residual errors are represented by e_{ij}. In our full model, fixed effects included *Test Number*, *Observation Number*, *Partner Experience* (experienced vs naïve), relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Test Number* and each relationship quality variable. By chance, five subjects were paired with experienced males (males that had previously been paired) and four subjects were paired with naïve males. While we did not have

particular predictions regarding how male experience would impact female behavior, we included *Partner Experience* in our fullest model for each outcome variable in case it impacted our subjects' behavior. *Partner Experience* never significantly explained variability in females' behavior, so ultimately it was not included in any of our final models. To determine the best-fitting model, we used backwards selection to remove any non-significant fixed effects (Bentler & Mooijaart, 1989). We used a log likelihood ratio test to compare model fit to determine whether removing any non-significant fixed effects resulted in a better fitting model (Vuong, 1989; Supplementary Table 1). We then performed a log likelihood ratio test to compare the fit of our best model to that of the null model where we removed all main effects (Vuong, 1989; West et al., 2012). The resulting final model, determined using a model comparison based on the likelihood of the model to the data, is the only one in which we evaluated the significance of the remaining parameters. Therefore, for each outcome variable, we had one final model where we interpreted significance level. The one final model represented the most likely hypothesized relationship between parameters given the data. Because we only evaluated parameter significance in one model per outcome, post hoc corrections were not necessary (Vuong, 1989; West et al., 2012). For all significant predictors we also calculated Cohen's f^2 as a measure of effect size (Selya et al., 2012; Snijders & Bosker, 1994). Based on Cohen's (J. Cohen, 1988) guidelines, $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively. We also had strong *a priori* hypotheses regarding how relationship quality variables and treatments would impact our outcome variables. We predicted that a higher-quality relationship with the father, as evidenced by greater distress upon separation and proximity maintenance, would result in greater proximity maintenance with the father during the first two

tests (1-week pre-pairing and 1-week post-pairing) and greater proximity maintenance with the partner 6-months post-pairing.

For Partner-directed Lip-smacking, Father-directed Lip-smacking, Back-arching/Tail-lashing, Chest Rubbing, and Long calling with the Father, because there were a large number of observations where females did not display these particular behaviors, we ran zero-inflated models with a Poisson distribution using the GLMMadaptive package (Rizopoulos, 2022). This allowed us to separately model the absence of a behavior and explain variability when females did display that behavior. In our fullest model, we included subject ID as a random effect for both the response and zero-part of the model. We also included the main effects of *Treatment*, *Test Number*, *Observation Number*, relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Treatment* and each attachment relationship quality. Similar to our methods for our LMM analyses, we used backwards selection to remove non-significant fixed effects and compared model fit using a log likelihood ratio test. The best-fitting model was compared to a null model, and parameter significance was only interpreted in that final model. We were unable to calculate Cohen's f^2 for our zero-inflated models.

Behaviors that were scored during testing but occurred too infrequently to model were Long call with Partner, Aggression towards Partner, Aggression towards Father, Receive Aggression from Partner, and Receive Aggression from Father.

Data Analysis Experiment 2

Data analyses for Experiment 2 were nearly identical to those conducted in Experiment 1. We checked for normality and transformed any non-normally distributed variables. We then conducted stepwise regression to identify which relationship quality variables we should include

in our LMM analyses. All LMM analyses included ID as a random, repeated measure. Our models were specified as: $y_{ij} = \beta_0 + \beta_1 x^{(1)}_{ij} + \cdots + \beta_m x^{(m)}_{ij} + \eta_{i0} + e_{ij}$. In this model, y_{ij} denotes the *j*th observation taken of individual *i* for outcome variable *y*. Individual covariates included in the model are represented by *x* (covariates 1-m), which have fixed effects common to all individuals in the population (β). This model also includes a random effect of ID that is specific to each individual (η_{i0}). Residual errors are represented by e_{ij}. In our full model for Social Salience Network, fixed effects included *Condition* (father, separated from father, partner, separated from partner), *Region* (amygdala, hypothalamus, LS, NAc, VP, VTA), *Side* (left, right), relationship quality variables (identified by previous stepwise regression analyses), and interaction effects between *Condition* and each relationship quality variable. LMM analyses for Whole Brain, and PAG were the same but did not include *Region* as a fixed effect. LMM analyses for cerebellum were the same as Whole Brain and PAG but did not include *Side* as a fixed effect. We used backwards selection and a log likelihood ratio test to identify the most parsimonious model that best explained variability in our data (Supplementary Table 2), and only interpreted significance from that one final model. We had strong *a priori* hypotheses regarding brain activity across our testing conditions. We predicted overall whole brain glucose uptake would be higher post-pairing compared to pre-pairing, while glucose uptake within the Social Salience Network, cerebellum, and PAG would decrease when females were separated from their current attachment figure (father when in the natal group and partner when paired) compared to when they were scanned with their attachment figure.

RESULTS

Experiment 1

During the 1-week pre-pairing and 1-week post-pairing tests, females spent more time in proximity to their fathers (Mean = -270.41, SD = 551.01, and Mean = -210.90, SD = 655.02, respectively; more negative scores indicate a greater preference for the father over the partner). However, 6-months post-pairing, females spent slightly more time in proximity to their partners (Mean = 58.47, SD = 562.49; more positive scores indicate a greater preference for the partner over the father), but this preference was less pronounced than females' preferences for their partners over strangers in other studies (Carp et al., 2016; Rothwell et al., 2020; Chapter Two Witczak, 2022).

Zone Ratio

We observed a large degree of variability with regards to where females spent time during preference testing (Figure 4). We wanted to disentangle these individual differences by first examining which zone females spent the most time in. We created a Zone Ratio score by multiplying the time females spent in their partner's preference zone by +1, the time in the father's preference zone by -1, and the time in the neutral zone by 0, and summing these three values per observation. Positive values represented more time in the partner's preference zone while negative values represented more time in the father's preference zone. Values closer to zero either indicated a lack of choice between the father and partner, with the female spending relatively the same amount of time in each zone, or a preference for the non-social areas of the testing arena.

The residuals for Zone Ratio scores were normally distributed; therefore, no transformations were needed. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, Infant Proximity, IOF Grate, IOF Locomotion, IOF Vocalizations, Juvenile Vocalizations, and Juvenile Locomotion* significantly predicted Zone Ratio scores. We then

compared a series of general linear mixed effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, only included the main effects of *Test Number* and *Juvenile Parent Preference* (Table 4a).

The R^2R^2 of this model is 0.1310. There was a significant, small effect of *Test Number* (f^2 = 0.067). Females spent significantly more time in proximity to their partners during test 3 compared to test 1 (β = 328.889, SE = 119.916, t = 2.743, p = .007; Figure 5a), and there was no difference between tests 1 and 2 (β = 59.511, SE = 119.916, t = 0.496, p = .621; Figure 5a). Females spent less time in proximity to their partner if they spent a greater proportion of time in their parents' proximity zone during juvenile testing (β = -7.780, SE = 2.278, t = -3.415, p < .001, f^2 = 0.087*;* Figure 5b), regardless of *Test Number*.

Percent Social

The Zone Ratio score does not distinguish between a female spending a lot of time in other and a female spending equal time in partner and father zones. To disentangle these two outcomes, we created a Percent Social score, which is the sum of time in the partner's preference zone plus the time in the father's zone divided by the sum of time spent in partner, father, and other. We then multiplied that value by 100. Larger values represent a greater percentage of time in a social zone, which would indicate a lack of choice between the father and partner if Zone Ratio is close to zero. Smaller values represent a greater proportion of time in the non-social areas of the testing arena. Across all three tests females spent the majority of the time in one of the two social preference zones (Mean = 69.99%, SD = 15.59%), and exhibited a significant

increase in time in the father and partner preference zones 6-months post-pairing (Mean = 74.64%, SD = 12.65%).

The residuals for Percent Social were normally distributed; therefore, no transformation was necessary. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, Juvenile Parent Choice, Infant Proximity, IOF Grate, IOF Locomotion, IOF Vocalizations,* and *Juvenile Vocalizations* significantly predicted Percent Social scores. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality behaviors. The best-fitting model, based on a log likelihood ratio test, did not include the main effects of *Observation Number* and *Partner Experience*; however, it did include all other main and interaction effects (Table 4b).

The R^2 of this model is 0.3573. There was a significant, large effect of *Test Number* on Percent Social (f^2 = 0.552). Females spent significantly more time in social zones of the preference testing arena during test 3 compared to test 1 (β = 7.708, SE = 2.794, t = 2.759, p = .007; Figure 6a), and there was no difference between tests 1 and 2 (β = 1.481, SE = 2.794, t = 0.530, p = .597; Figure 6a). Females spent less time in social zones if they vocalized more during IOF testing when separated from their fathers (β = -0.191, SE = 0.096, t = -1.983, p = .050, f^2 < .001; Figure 6b). Interestingly, if females vocalized more when separated from their fathers during juvenile social separation testing, they spent more time in the social preference zones as adults (β = 0.238, SE = 0.084, t = 2.847, p = .005, f^2 < .001; Figure 6c). Finally, we found one significant interaction effect. For females that were carried more by their fathers as infants, they

spent less time in the social zones during test number 2 compared to test number 1 (β = -3.030, SE = 1.521, t = -1.992, p = .050, f^2 = 0.030; Figure 6d).

Partner Zone

We were also interested in understanding what factors explain variability in the amount of time females spent within a particular preference zone; therefore, we examined duration of time in each zone separately. The residuals for partner preference zone were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity*, *Juvenile Parent Preference*, and *Juvenile Parent Choice* significantly predicted partner preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number*, and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, only included the main effects of test number, *Juvenile Proximity, Juvenile Parent Preference*, and *Juvenile Parent Choice* (Table 4c).

The R^2 of this model is 0.2580. There was a significant, medium effect of *Test Number* on partner preference zone duration (f^2 = 0.333). There was no significant difference in time spent in the partner's zone between tests 1 and 2 (β = 0.521, SE = 1.357, t = 0.384, p = .701; Figure 7a); however, females spent significantly more time in their partner's preference zone during test 3 (β = 5.196, SE = 1.357, t = 3.828, p < .001; Figure 7a). Females that spent more time in proximity to their fathers during the reunion period following social separation testing as juveniles also spent more time in proximity to their partner during these adult preference tests (β = 0.060, SE = 0.021, t = 2.853, p = .005, f^2 = 0.062; Figure 7b). Interestingly, females that spent more time in proximity to their parents during juvenile preference testing spent less time in

proximity to their partners during this adult testing (β = -0.155, SE = 0.030, t = -5.167, p < .001, f^2 = 0.207; Figure 7c). However, females that choose their parents more during catch and release as juveniles spent more time in proximity to their partners during this adult test (β = 0.101, SE = 0.036, t = 2.806, p = .006, f^2 = 0.060; Figure 7d).

Father Zone

Residuals for father preference zone duration were normally distributed following a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Parent Preference,* and *Juvenile Locomotion* significantly predicted father preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number, and Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, only included the main effects of *Observation Number* and *Juvenile Parent Preference* (Table 4d).

The R^2 of this model is 0.1089. We did not find evidence to suggest that the amount of time females spent in proximity to their fathers changed across the three tests (Figure 8a). Females spent less time in proximity to their fathers as observation number increased from the first (Mean = 765.37 seconds, SD = 287.23 seconds) to the last (Mean = 618.78 seconds, SD = 299.15; β = -0.811, SE = 0.373, t = -2.173, p = .032, f^2 = 0.068; Figure 8b). No other predictors significantly explained time spent in proximity to the father. There was a non-significant trend towards females spending more time in proximity to their fathers if they also spent more time in proximity to their parents during juvenile preference testing ((β = 0.055, SE = 0.032, t = 1.710, p = .131, f^2 = 0.068; Figure 8c).

Other Zone

Any time females spent outside of the partner or father's preference zone was considered time spent in "other". We wanted to understand what factors explained variability in the amount of time females spent in the non-social parts of the testing arena. The residuals for other preference zone duration were not normally distributed so we used a square-root transformation. Based on stepwise regression analyses, the variables *Juvenile Parent Preference, Infant Proximity, IOF Grate, IOF Locomotion, IOF Vocalizations, Juvenile Vocalizations,* and *Juvenile Locomotion* significantly predicted other preference zone duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included the main effects of *Test Number* and all tested relationship quality variables with no interaction effects (Table 4e).

The R^2 of this model is 0.2340. We found a significant, moderate effect of *Test Number* on other zone duration (f^2 = 0.301). Females spent less time in the non-social "other" areas of the preference testing arena in test 3 compared to test 1 (β = -2.952, SE = 1.184, t = -2.493, p = .014; Figure 9a). Females spent less time in other if they spent a greater proportion of time in their parents' proximity zone during juvenile testing (β = -0.101, SE = 0.044, t = -2.319, p = .022, f^2 = 0.055; Figure 9b). Females also spent less time in other if they vocalized more when separated from their fathers during the saline condition of juvenile social separation testing (β = -0.047, SE = 0.020, t = -2.281, p = .024, f^2 = 0.053; Figure 9c). While our best-fitting model accounted for

the effects of other relationship quality variables, none significantly explained variability in time spent in the non-social areas of the preference test arena.

Partner Grate Touching

When females were within the partner or father preference zones, they could interact at the grate, which allowed the closest access to the stimulus animal possible. We wanted to know what variables predicted differences in grate-touching behavior in our subjects. The residuals for partner grate touch duration were not normally distributed so we used a cube-root transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Preference, IOF Locomotion, Juvenile Vocalization, and Pair Affiliation* significantly predicted partner grate touch duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included *Test Number,* the main effects of all tested relationship quality variables, and an interaction between *Test Number* and *Juvenile Parent Preference* (Table 4f).

The R^2 of this model is 0.3552. We did not find a significant effect of *Test Number* on partner grate touch duration (Figure 10a). None of the relationship quality related variables significantly explained partner grate touching duration on their own; however, there was a significant, moderate interaction effect between *Test Number* and *Juvenile Parent Preference* (f^2 = 0.161). Specifically, during test 2, females that spent a greater amount of time in their parents' preference zone during juvenile testing spent less time touching the partner's grate (β = -0.037, SE = 0.011, t = -3.401, $p < .001$; Figure 10b).

Father Grate Touching

The residuals for father grate touch duration were not normally distributed so we used a cube-root transformation. Based on stepwise regression analyses, the variables *Infant Proximity, IOF Proximity, IOF Vocalizations, Juvenile Vocalizations*, *Juvenile Locomotion,* and *Parent Affiliation* significantly predicted father grate touch duration. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included *Test Number, Observation Number,* all main effects of relationship quality variables, and interaction effects between *Test Number* and *IOF Proximity, IOF Vocalization,* and *Parent Affiliation* (Table 4g).

The R^2 of this model is 0.4491. We did not find a significant effect of *Test Number* on father grate touch duration (Figure 11a). However, there was a significant, small effect of *Observation Number* such that females spent less time touching their fathers' grate as *Observation Number* increased from one to five ($\beta = -0.260$, SE = 0.064, $t = -4.043$, $p < .001$, $f^2 = 0.099$; Figure 11b). None of the relationship quality-related variables significantly explained partner grate touching duration on their own; however, there was a significant, small interaction effect between *Test Number* and *IOF Proximity* ($f^2 = 0.102$). Specifically, during test 3, females that exhibit a greater increase in time in proximity to their father during IOF testing spend more time touching the father's grate ($\beta = -0.030$, SE = 0.009, $t = 3.184$, $p = .002$; Figure 11c). We also found a significant, small interaction effect between *Test Number* and *IOF Vocalizations* ($f^2 = 0.051$). Females that exhibit a greater increase in vocalizations when separated from their

father during IOF testing spend more time touching their father's grate during test 2 (β = 0.008, SE = 0.003, t = 2.156, p = .033; Figure 11d).

Zone Change Frequency

We used the number of times females changed between the partner, father, and other preference zones as a measure of general locomotion during testing. Residuals for Zone Change Frequency were normally distributed following a log transformation. Based on stepwise regression analyses, the variables *Juvenile Parent Choice, Juvenile Locomotion,* and *Parent Affiliation* significantly predicted females' zone change frequency. We then compared a series of general linear mixed-effects models with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve). ID was included as a random, repeated measure. *Test Number* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, was the model only including the main effects of *Test Number* and the three tested relationship quality variables fit best (Table 4h).

The R^2 of this model is 0.3761. We found a significant, large effect of *Test Number* on Zone Change Frequency (f^2 = 0.590). Females changed preference zones significantly less during test number 2 compared to test number 1 (β = -0.484, SE = 0.013, t = -3.700, p < .001; Figure 12a). Females also changed preference zones less if they chose their parents a greater percentage of time during the saline condition of catch and release during juvenile preference testing (β = -0.014, SE = 0.003, t = -4.431, p = .007, f^2 = 0.169; Figure 12b). Females changed zones more if they locomoted more during the saline condition of juvenile social separation testing (β = 0.0007, SE = 0.00002, t = 3.791, p = .013, f^2 = 0.124; Figure 12c). Females changed zones less if

their parents spent a greater percentage of their time in social affiliation while females were in the natal group (β = -0.041, SE = 0.007, t = -5.165, p = .004, f^2 = 0.227; Figure 12d).

Partner-Directed Lip-smacking

We observed a large degree of variability in lip-smacking behavior between females and across tests. Some females hardly ever lip-smack while others lip-smack frequently. We examined when females do and do not lip-smack in the same model to fully capture this variability. Based on stepwise regression analyses, the variables *IOF Locomotion* and *IOF Vocalizations* significantly predicted partner-directed lip-smacking. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve) for both the response and zero-part of the models. ID was included as a random, repeated measure for both the response and zero-part of the model in the fullest model. *Test Number* was allowed to interact with all relationship quality-related behaviors in both parts of the model. The best-fitting model, based on a log likelihood ratio test, was the model only including the main effect of *Test Number* for the zero-part of the model (no random effect), and the main effects of *Test Number, Observation Number, IOF Locomotion, IOF Vocalization,* plus the interaction between *Test Number* and *IOF Vocalization* (Table 4i).

Based on the results of this best-fitting model, females lip-smacked at their partners more in test 2 (β = 0.652, SE = 0.213, Z = 3.058, p = .002; Figure 13a) and test 3 (β = 0.504, SE = 0.218, Z = 2.313, p = .021; Figure 13a) compared to test 1 (when the partner was a stranger). Females also lip-smacked at their partners less as observation number increased from one to five (β = -0.289, SE = 0.034, Z = -8.591, p < .001; Figure 13b). Females lip-smacked at their partners less if they exhibited a greater increase in line-crossing during IOF testing in the alone condition

compared to the father condition (β = -0.005, SE = 0.001, Z = -3.688, p < .001; Figure 13c). There was a significant interaction effect between test number and IOF Vocalizations. Specifically, females lip-smacked at their partners more in test 3 (β = 0.025, SE = 0.004, Z = 5.884, p < .001; Figure 13d) but less in test 2 (β = 0.011, SE = 0.004, Z = 2.654, p = .008; Figure 13d) if they exhibited a greater increase in vocalizations when separated from their father during IOF testing.

Father-Directed Lip-smacking

Based on stepwise regression analyses, the variables *Juvenile Parent Preference, IOF Proximity,* and *Pair Affiliation* significantly predicted father-directed lip-smacking. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve) for both the response and zero-part of the models. ID was included as a random, repeated measure for both the response and zero-part of the model in the fullest model. *Test Number* was allowed to interact with all relationship quality-related behaviors in both parts of the model. The best-fitting model, based on a log likelihood ratio test, was the model that included random effects for both the response and zero-part of the model along with the main effect of *Test Number* for the zero-part and all possible main and interaction effects (except for *Partner Experience*) in the response-part of the model (Table 4j).

Test Number did not significantly explain variability in father-directed lip-smacking (Figure 14a). Based on the results of this best-fitting model, females lip-smack at their fathers significantly less as observation number increases from one to five (β = -0.527, SE = 0.041, Z = -12.778, p < .001; Figure 14b). No other predictors significantly explained variance in father-

directed lip-smacking behavior, despite the best-fitting model accounting for additional relationship quality variables.

Back-arching/Tail-lashing

Back-arching/tail-lashing was another behavior that was highly variable between subjects and across tests. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Preference, Juvenile Parent Choice, IOF Locomotion,* and *IOF Vocalizations* significantly predicted back-arching/tail-lashing. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve) for both the response and zero-part of the models. ID was included as a random, repeated measure for both the response and zero-part of the model in the fullest model. *Test Number* was allowed to interact with all relationship quality-related behaviors in both parts of the model. The best-fitting model, based on a log likelihood ratio test, was the model that included random effects for the response part but not the zero-part of the model. The zero-part of the model was significantly predicted by the main effects of *Observation Number, Partner Experience, Juvenile Proximity, Juvenile Parent Preference,* and *IOF Vocalizations*. The response-part of the model included the main effects of *Test Number, Observation Number,* all relationship quality variables tested, and all interactions between relationship quality variables and test number (Table 4k).

Based on the results of this best-fitting model, females arched/lashed more in test 2 (β = 0.575, SE = 0.161, Z = 3.578, $p < .001$; Figure 15a) and test 3 (β = 0.950, SE = 0.146, Z = 6.520, $p < .001$; Figure 15a) compared to test 1. Within a test, females arched less as the observation number increased from one to five (β = -0.472, SE = 0.024, Z = -19.461, $p < .001$; Figure 15b), and were more likely to not arch as observation number increased (β = 0.552, SE = 0.207, Z =

2.668, p = .007). When examining the results for the zero-part of the model, females are less likely to not arch/lash when their partners are naïve (β = -5.422, SE = 1.712, Z = -3.167, p = .002), suggesting females arch more in response to naïve males. Females are also less likely to not arch if they spend a greater proportion of time in contact with their fathers during the reunion period following juvenile social separation testing (β = =0.072, SE = 0.020, Z = -3.615, p < .001). Similarly, females are less likely to not arch/lash if they exhibit a greater increase in vocalizations from the father to the alone condition during IOF testing (β = -0.049, SE = 0.014, Z = -3.607, p < .001). Interestingly, females are more likely to not arch/lash if they spent a greater proportion of time in proximity to their parents during juvenile preference testing (β = 0.096, SE = 0.032, Z = 2.930, p = .003).

When examining the results for the response part of the model, we found several significant interaction effects between relationship-quality variables and test number. Females that spend a greater percentage of time in proximity to their fathers during the reunion period following juvenile social separation testing arch/lash significantly less during test 3 compared to test 1 (β = -0.020, SE = 0.007, Z = -2.752, p = .005; Figure 15c). Females that spend a greater percentage of time in their parents' preference zone during juvenile PPTs arch/lash more during tests 2 (β = 0.053, SE = 0.012, Z = 4.474, p < .001; Figure 15d) and 3 (β = 0.047, SE = 0.012, Z = 3.816, p < .001; Figure 15d). Females that choose their parents more during catch and release arch/lash more during tests 2 (β = -0.026, SE = 0.013, Z = -2.025, p = .043; Figure 15e) and 3 (β = -0.049, SE = 0.012, Z = -3.956, p < .001; Figure 15e). Similarly, females that line cross more in the alone condition compared to the father condition of IOF testing arch/lash more in tests 2 (β = 0.007, SE = 0.002, Z = 3.927, p < .001; Figure 15f) and 3 (β = 0.009, SE = 0.002, Z = 5.495, p < .001; Figure 15f). Finally, females that vocalize more when separated from their father during

IOF testing also arch/lash more during tests 2 (β = -0.010, SE = 0.003, Z = -3.434, p < .001; Figure 15g) and 3 (β = -0.008, SE = 0.003, Z = -2.646, p = .008; Figure 15g).

Chest Rubbing

Chest-rubbing was another variable behavior between subjects and across tests. Based on stepwise regression analyses, the variables *IOF Grate* and *Pair Affiliation* significantly predicted chest-rubbing. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve) for both the response and zero-part of the models. ID was included as a random, repeated measure for both the response and zero-part of the model in the fullest model. *Test Number* was allowed to interact with all relationship quality-related behaviors in both parts of the model. The best-fitting model, based on a log likelihood ratio test, was the model that included random effects for the response part but not the zero-part of the model. The zero-part of the model was significantly predicted by the main effects of *Test Number, IOF Grate, Pair Affiliation,* and the interaction between *Test Number* and *IOF Grate*. The response-part of the model included the main effects of *Test Number, Observation Number*, both relationship quality variables tested, and both interactions between relationship quality variables and *Test Number* (Table 4l).

Based on the results of this best-fitting model, when females did chest-rug, they did not significantly differ in their frequency across the three tests (Figure 16a). However, females were more likely to chest rub in test 3 compared to test 1 (β = -2.272, SE = 0.790, Z = -2.877, p = 0.004). Females also tended to chest-rub less as observation number increased from one to five (β = -0.139, SE = 0.046, Z = -3.050, p = 0.002; Figure 16b). With regards to explanations for zero responses of chest-rubbing, females were less likely to not chest rub if they spent a greater

percentage of time in affiliative contact with their partners during the first six months of pairing (β = -0.220, SE = 0.045, Z = -4.858, $p < .001$). Females were more likely to not chest-rub during test 3 if they spent a greater amount of time touching their father's grate compared to the empty box grate during IOF testing (β = 0.108, SE = 0.049, Z = 2.218, p = 0.027).

Upon examination of the results for the response-part of the model, we observed two significant interaction effects. Females that spend a greater percentage of time touching the grate during IOF testing when with their father compared to the empty box condition chest rub more during test 2 (β = 0.062, SE = 0.020, Z = 3.094, p = 0.002; Figure 16c) compared to test 1. Females that spend a greater percentage of time in affiliative contact with their partners in the home-cage during the first six months of pairing chest-rub more during test 3 (β = 0.108, SE = 0.049, Z = 2.218, p = 0.027; Figure 16d).

Long calling with Father

The final behavior we were able to analyze for Experiment 1 was females' long calling with their fathers during preference testing. This behavior was also highly variable between subjects and across tests, so we used models to account for both presence and absence of long calling with the father. Based on stepwise regression analyses, the variables *IOF Grate* and *IOF Locomotion* significantly predicted long calling with the father. We then compared a series of zero-inflated mixed-effects models with a Poisson distribution with different combinations of these variables with *Test Number, Observation Number,* and *Partner Experience* (experienced vs naïve) for both the response and zero-part of the models. ID was included as a random, repeated measure for both the response and zero-part of the model in the fullest model. *Test Number* was allowed to interact with all relationship quality-related behaviors in both parts of the model. The best-fitting model, based on a log likelihood ratio test, was the model that included random

effects for the response part but not the zero-part of the model. The zero-part of the model was significantly predicted by the main effects of *Observation Number* and *IOF Locomotion*. The response-part of the model included the main effects of *Test Number, Observation Number*, both relationship quality variables tested, and both interactions between relationship quality and *Test Number* (Table 4m).

Based on the results of this best-fitting model, females were more likely to not long call with their fathers as observation number increased from one to five ($\beta = 0.519$, SE = 0.213, Z = 2.434, p = .015). Females were less likely to not long call with their father if they exhibited a greater increase in line-crossing during IOF testing ($\beta = -0.004$, SE = 0.002, Z = -2.204, p = .028). When females did long call with their fathers, they long called significantly more during test 3 compared to test 1 ($\beta = 0.646$, SE = 0.286, Z = 2.260, p = .024; Figure 17a). We also found two significant interaction effects. Females that spend more time touching their father's grate compared to the empty box grate during IOF long called with their father more during test number 2 ($\beta = -0.068$, SE = 0.028, Z = -2.445, p = .014; Figure 17b). Females that exhibited a greater increase in line-crossing during IOF long called with their father more during test number 2 ($\beta = 0.005$, SE = 0.002, Z = 3.046, p = .002; Figure 17c).

Experiment 2

Social Salience Network

Residuals were normally distributed for social salience network activity following a log-transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity* and *Parent Affiliation* significantly predicted social salience network activity. We then compared a series of general linear mixed effects models with different combinations of these variables with *Condition* (father, separated from father, partner, separated from partner), *Region* (amygdala,

hypothalamus, LS, NAc, VP, VTA), and *Side* (left, right). ID was included as a random, repeated measure. *Condition* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for *Side* (Table 5a).

The R^2 of this model is 0.9712. We found a significant, large effect of condition on social salience network activity (f^2 = 0.751). When examining the social salience network as a whole, compared to when females are scanned with their fathers (baseline), females exhibit less total activity when scanned with their partner (β = -0.365, SE = 0.034, t = -10.855, p < .001; Figure 18a), and when separated from their father (β = -0.074, SE = 0.034, t = -2.210, p = .028; Figure 18a) and separated from their partner (β = -0.421, SE = 0.034, t = -12.494, p < .001; Figure 18a).

We ran a Tukey's post-hoc comparison to compare activity across the six brain regions and found that the individual regions within the social salience network all differed in their total activity (Table 6). The amygdala had the highest amount of activity in comparison to all other brain regions, followed by the NAc, then the hypothalamus (Table 6; Figure 18a). The VP had the least amount of activity, relative to the other regions, followed by the VTA and the LS (Table 6; Figure 18a).

With regards to the effects of relationship quality on total activity, we found significant interaction effects between condition and relationship quality. Specifically, *Juvenile Proximity* is negatively related to Total Activity when females are tested with their partner (β = -0.005, SE = 0.001, t = -4.854, p < .001; Figure 18b) and separated from their father (β= -0.012, SE = 0.001, t = -10.218, p < .001; Figure 18b), suggesting females that spend a greater percentage of time in proximity to their fathers following a separation exhibit lower activity of the social salience network during these two test conditions (f^2 = 0.228). We found a positive relation between

Parent Affiliation and Total Activity when females were with their partners (β = 0.022, SE = 0.005, t = 4.787, p < .001; Figure 18c) and separated from their partners (0.017, SE = 0.005, t = 3.718, p < .001; Figure 18c), suggesting they exhibited higher activity of the social salience network during these two conditions if their parents spent a greater percentage of time in affiliative contact when they were juveniles (f^2 = 0.096).

Whole Brain

A log transformation using base e ($\log_e$ transformation) resulted in normal distribution of the residuals for whole brain activity. Based on stepwise regression analyses, the variables *Juvenile Proximity*, *Juvenile Parent Choice*, *IOF Proximity*, and *IOF Locomotion* significantly predicted whole brain activity. We then compared a series of general linear mixed effects models with different combinations of these variables with *Condition* (father, separated from father, partner, separated from partner) and *Side* (left, right). ID was included as a random, repeated measure. *Condition* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included all main and interaction effects except for *Side* (Table 5b).

The R^2 of this model is 0.7293. We found a significant, large effect of condition on social salience network activity (f^2 = 2.607). Whole brain activity was lower when females were tested with their partner (β = -0.378, SE = 0.074, t = -5.098, p < .001; Figure 19a) and when they were separated from their partner (β = -0.411, SE = 0.074, t = -5.540, p < .001; Figure 19a) compared to when they were tested with their father while in their natal group. We found significant interaction effects between *Condition* and two of the relationship quality variables. Specifically, we found a negative relation between *Juvenile Proximity* and Total Activity when females were tested with their partners (β = -0.007, SE = 0.003, t = -2.354, p = .023; Figure 19b) and separated

from their fathers (β = -0.014, SE = 0.003, t = -4.484, p < .001; Figure 19b), suggesting females that spend a greater amount of time in proximity to their fathers following juvenile separation testing exhibit lower whole brain activity in these two conditions (f^2 = 0.342). We found a negative relation between *Juvenile Parent Choice* and Total Activity when females were separated from their partners (β = -0.010, SE = 0.004, t = -2.267, p = .028; Figure 19c), suggesting females that choose their parents a greater percentage of time during catch and release testing have lower whole brain activity when separated from their partners (f^2 = 0.109).

Cerebellum

We used a $\log_e$ transformation to normalize the distribution of the residuals for cerebellum activity. Based on stepwise regression analyses, the variables *Juvenile Proximity* and *Parent Affiliation* significantly predicted cerebellum activity. We then compared a series of general linear mixed effects models with different combinations of these variables with *Condition* (father, separated from father, partner, separated from partner). ID was included as a random, repeated measure. *Condition* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included all main effects and an interaction between *Condition* and *Juvenile Proximity* (Table 5c).

The R^2 of this model is 0.5588. We found a significant, large effect of condition on cerebellum activity (f^2 = 1.266). Cerebellum activity was lower when females were tested with their partner (β = -0.378, SE = 0.074, t = -5.098, p < .001; Figure 20a) and when they were separated from their partner (β = -0.411, SE = 0.074, t = -5.540, p < .001; Figure 20a) compared to when they were tested with their father while in their natal group. We found a significant, medium size interaction effect between *Condition* and *Juvenile Proximity* (f^2 = 0.239). Specifically, we found a negative relation between *Juvenile Proximity* and Total Activity when

females were tested separated from their fathers (β = -0.012, SE = 0.004, t = -2.770, p = .010; Figure 20b), suggesting females that spend a greater amount of time in proximity to their fathers following juvenile separation testing exhibit lower cerebellum activity when separated from their father.

Periaqueductal Gray (PAG)

The residuals for PAG activity were normally distributed following a $\log_e$ transformation. Based on stepwise regression analyses, the variables *Juvenile Proximity, Juvenile Parent Choice, IOF Proximity, IOF Grate, Juvenile Vocalization,* and *Parent Affiliation* significantly predicted PAG activity. We then compared a series of general linear mixed effects models with different combinations of these variables with *Condition* (father, separated from father, partner, separated from partner) and *Side* (left, right). ID was included as a random, repeated measure. *Condition* was allowed to interact with all relationship quality-related behaviors. The best-fitting model, based on a log likelihood ratio test, included all main effects except for *Side* in addition to interactions between *Condition* and *Juvenile Proximity, IOF Grate, Juvenile Vocalization,* and *Parent Affiliation* (Table 5d).

The R^2 of this model is 0.8253. There was a significant, large effect of condition on PAG activity (f^2 = 3.110). PAG activity was lower when females were tested with their partner (β = -0.363, SE = 0.060, t = -6.071, p < .001; Figure 21a) and when they were separated from their partner (β = -0.442, SE = 0.060, t = -7.403, p < .001; Figure 21a) compared to when they were tested with their father while in their natal group. We found several significant interaction effects between *Condition* and relationship quality variables. Specifically, we found a negative relation between *Juvenile Proximity* and Total Activity when females were tested separated from their fathers (β = -0.009, SE = 0.003, t = -3.207, p = .002; Figure 21b), suggesting females that spend

a greater amount of time in proximity to their fathers following juvenile separation testing exhibit lower PAG activity when separated from their father (f^2 = 0.234). Interestingly, we found opposite relations between *IOF Grate* and Total Activity depending on whether females were tested with their partner or separated from their partner (f^2 = 0.275). If females spent a greater percentage of time touching their father's grate during IOF testing as infants, they had lower PAG activity when tested with their partner (β = 0.010, SE = 0.005, t = 2.090, p = 0.041; Figure 21c) but higher PAG activity when separated from their partner (0.022, SE = 0.005, t = 4.757, p < .001; Figure 21c). Interactions between *Condition* and *Juvenile Vocalizations* also significantly explained variability in PAG activity (f^2 = 0.240). Specifically, we found a positive relation between *Juvenile Vocalization* and Total Activity when females were tested with their partner (β = 0.006, SE = 0.002, t = 4.244, p < .001; Figure 21d) and separated from their father (β = 0.003, SE = 0.002, t = 2.203, p = .032; Figure 21d), but a negative relation when females were separated from their partner (β = 0.006, SE = 0.002, t = 3.938, p < .001; Figure 21d). These findings suggest females that vocalize more when separated from their fathers exhibit greater PAG activity when with their partners and separated from their fathers, but lower PAG activity when separated from their partner. Finally, we found a significant interaction between *Condition* and *Parent Affiliation* (f^2 = 0.422). Females exhibit greater PAG activity when tested with their partners (β = 0.044, SE = 0.011, t = 4.082, p < .001; Figure 21e) and separated from their partners (β = 0.054, SE = 0.011, t = 5.058, p < .001; Figure 21e) if their parents spent a greater percentage of time in affiliative contact when subjects were infants and juveniles.

DISCUSSION

To study how relationship quality impacts the behavioral and neural correlates of social bonds, we conducted two experiments in parallel. Experiment 1 investigated how the quality of a

daughter's relationship with her father influenced proximity maintenance behaviors between her father and her pair bonded partner. Experiment 2 investigated how relationship quality affects glucose uptake when females are temporarily separated from their current attachment figure (the father while in the natal group and the partner 6-months post-pairing). In general, we found that measures of relationship quality significantly explained variability in female proximity maintenance behaviors and neural activity.

Experiment 1

At the start of our preference testing experiment, females were an average of 28.62 months of age (SD = 0.51 months) one week before they were paired with a partner. By the end of Experiment 1, when females had been paired with their partners for six months, females were on average 35.36 months of age (SD = 0.44 months). This age range captures when female titi monkeys begin regularly cycling (Conley et al., 2022) and leave their natal groups to find a partner in the wild (Bossuyt, 2002; Souza-Alves et al., 2019; Thompson & Norconk, 2011; Van Belle et al., 2016). Therefore, all females were capable of forming monogamous pair bonds and likely would have done so at this time in the wild. Six months after pairing male and female titi monkeys exhibit a strong preference for their partner over a stranger (Rothwell et al., 2020), and captive titi monkey pairs reach peak affiliation levels 6-months post-pairing based on scan sampling data (Witczak, Blozis, & Bales, 2022). What is less clear is how parent-offspring relationships change as offspring form and maintain adult pair bonds.

In the wild, titi monkeys do not emigrate far from their natal groups (Dolotovskaya, Roos, & Heymann, 2020), and therefore it is important to understand how attachments shift from the father to the partner when females are able to interact with both. In the wild, offspring do not appear to leave their natal groups due to aggression from family members (Bossuyt, 2002;

Souza-Alves et al., 2019; Thompson & Norconk, 2011; Van Belle et al., 2016) and offspring have been observed reassimilating into natal groups following a one-month separation in captivity (Hoffman, 1996); therefore, there must be some factor that primes offspring to leave their father for the unfamiliar conspecific that becomes their partner.

In humans, the quality of relationship between and parent and child impacts adult romantic relationships of those children (River et al., 2022), and it is thought that parent-child relationships are particularly influential for first relationships (Dinero et al., 2008). Therefore, we investigated how the quality of relationship a daughter has with her father impacts the daughter's proximity maintenance behaviors with her father and partner from when pair bonds are just forming to when pair bonds have been firmly established. The present study is the first to directly examine proximity maintenance behaviors in females when they are given a choice between their previous primary attachment figures, their fathers, and their current pair bonded partner. In contrast to previous findings comparing proximity maintenance with strangers compared to former and current attachment figures, where females significantly prefer their current partner over strangers and show no preference between their previous attachment figure (their father) and strangers (Carp et al., 2016), females in the present study exhibited less of a clear preference between the father and partner. In particular, when looking at Zone Ratio scores, which captured preference between the partner and the father, scores were much closer to zero than they are for tests when subjects are given a choice between their parents and strangers (Mean = 528.61, SD = 567.65; more positive scores indicate greater preference for the parents over strangers; Chapter Two Witczak, 2022). During the 1-week pre-pairing and 1-week post-pairing tests, females spent more time in proximity to their fathers. However, 6-months post-pairing, females spent slightly more time in proximity to their partners, but this preference was

less pronounced than females' preferences for their partners over strangers in other studies (Carp et al., 2016; Rothwell et al., 2020; Witczak, 2022). Even though these females had been separated from their fathers for six months with no contact, they still appeared to maintain some sort of attachment to the father. What was particularly interesting was that females that had previously spent a greater proportion of time in proximity to their parents during juvenile parent preference tests spent less time with their partners compared to their fathers across all three time points. These findings suggest that females with a higher-quality relationship with their father may be more likely to maintain that relationship with their father, even after they have formed a pair bond with an adult conspecific. It would be interesting to study how long this attachment to the father persists and how it might impact interactions between pairs in the wild.

Our Zone Ratio score cannot differentiate between a subject spending equal amounts of time with the partner and father and a subject spending the majority of their time in the non-social parts of the testing arena; therefore, we also calculated a Percent Social score to determine whether a Zone Ratio score closer to zero indicated equal time between stimulus animals or more time away from either stimulus animal. Across all three tests females spent the majority of the time in one of the two social preference zones, and exhibited a significant increase in time in the father and partner preference zones 6-months post-pairing. We did find some significant effects of relationship quality on time spent in the social zones of the test, however, their effect sizes were very small ($f^2 < 0.001$), so it is difficult to conclusively interpret these results. It is possible that this Percent Social score is not the best for disentangling variability in proximity maintenance behavior, as all females were highly motivated to engage socially with their fathers and partners during this test.

To better understand variability in time spent with specific social partners, we examined the effects of daughters' relationship quality on time spent in each preference zone: partner, father, and other. Females spent significantly more time in their partner's preference zone at the 6-month post-pairing time point, but variability in time spent maintaining proximity with the partner was affected by the daughter's quality of relationship with her father. Regardless of the testing time point (pre-pairing, 1-week post-pairing, or 6-months post-pairing), females spent more time in proximity to their partner if they scored higher on measures of *Juvenile Proximity* and *Juvenile Parent Choice.* Therefore, these measures of relationship quality predicted an increased willingness to form and maintain an adult pair bond. It is possible that this higher-quality relationship with the father set the foundation for females to develop high-quality relationships with their partners. Indeed, a previous study in titi monkeys similarly found that infant attachment was related to adult pair mate affiliation (Savidge & Bales, 2020). While our measures of infant relationship quality did not significantly explain variability in proximity maintenance behaviors during this preference test paradigm, juvenile measures of relationship quality did. Interestingly, we found the opposite relation between *Juvenile Parent Preference* and time in the partner's preference zone, where females that spent more time in their parents' preference zone in previous tests spent less time in proximity to their partners. This finding may be less of a reflection of a poorer relationship with their partner and instead might represent a greater ability to maintain a previous relationship while establishing a new one. Therefore, our separate measures of relationship quality may differentially explain females' ability to form new relationships and maintain older attachments. It has previously been thought that titi monkeys, like many non-human animals, can only maintain one selective attachment at a time (Bales et al., 2021); however, these findings suggest titi monkeys have the potential to somewhat maintain

aspects of old relationships, such as the desire to maintain proximity to their father, after they form a new relationship. Future studies should investigate how long after pair formation females continue to maintain an attachment to their father, particularly in the wild when they may have more opportunities to interact with their fathers (Dolotovskaya, Roos, et al., 2020).

While relationship quality with the father explained variability in proximity maintenance with the partner, only observation number explained variability in time spent in proximity to the father during testing. Specifically, females exhibited a slight decrease in time spent in proximity to the father as observations increased from the first to the last within a test. These findings indicate differences in initial reactions to reunion with the father compared to prolonged interactions. While females may be more likely to spend time in close proximity to their fathers when they are first reunited with them, it would not be expected for them to maintain a preference for remaining close to their father over their partner. This shift in proximity behavior over time may allow females to maintain their current pair bond while also still maintaining some relationship with their father after they have left the natal group.

While females did spend the majority of their time in the social preference zones during testing, we did see some variability in how much time was spent in the non-social areas of the test. Females spent less time in the non-social areas 6-months post-pairing, and across all tests. Measures of juvenile proximity maintenance and separation distress were negatively related to time in other, suggesting females with higher-quality relationships with their fathers were more willing to engage socially during this testing paradigm. The mechanism underlying this relationship are still under investigation, but a previous study in titi monkey juveniles found evidence that OT and AVP may be involved in heightened female titi monkey sociality (Chapter Two: (Witczak, 2022). It is possible that differences in relationship quality may alter OT and

AVP systems in such a way that makes them more responsive to social cues. OT receptor binding has been demonstrated to differ in the hippocampal formation based on levels of affiliation observed between pair-mates in titi monkeys (Baxter et al., 2020). Given the relationship between OT, AVP, and the HPA-axis (Gobrogge & Wang, 2015; Spencer & Deak, 2016), it is possible that higher-quality social bonds may alter OT and AVP activity in such a way that it reduces feelings of stress and anxiety in the presence of others, promoting higher social engagement.

A previous titi monkey study found differences between preference zone duration and grate touching behavior, particularly when subjects were presented with their father and a stranger (Carp et al., 2016); therefore, we wanted to investigate how female relationship quality impacted this specific proximity maintenance behavior. While variability in where females spent time during testing was not significantly explained by interactions between *Test Number* and relationship quality, the specific behaviors we investigated, including grate touching, did differ by test number based on differences in measures of female relationship quality. With regards to partner grate-touching behavior, females spent less time touching their partner's grate if they exhibited higher preference for maintaining proximity to their parents as juveniles, but only for the 1-week post-pairing test. Therefore, immediately following separation from their family, females with a higher-quality relationship with their father are less likely to engage with a new partner while they are still forming their pair bond. This finding suggests that the transition from being primarily attached to the father to selectively bonded to the partner is not immediate, particularly for females that have a higher-quality relationship with their fathers. Interestingly, when examining partner grate touching behavior, infant measure of proximity maintenance and separation distress correlated with greater father grate touching in the 6-month post-pairing and

1-week post-pairing tests, respectively. As with patterns of proximity maintenance, these findings suggest females with higher-quality relationships may be better able to maintain multiple relationships, even when they have formed an established pair bond.

We also measured how frequently females moved between preference zones to determine how stable their proximity maintenance patterns were. We found that females changed preference zones less frequently if they chose their parents more during juvenile catch and release testing and if their parents spent more time in affiliative contact during the first 14 months of the female's life. These findings suggest that both the *experience* of a high-quality relationship and the *observation* of a high-quality relationship may influence how stable a females' choice is in terms of who she prefers to spend time in proximity to. Research in humans has demonstrated how parent-child relationship quality impacts adult romantic relationships (for a review, see River et al., 2022); however, the effect of parents' relationship quality with each other on their offspring's later romantic relationships is less well understood and warrants further investigation. Studies that have evaluated this connection have primarily focused on the negative consequences of divorce and conflict between parents (Gager et al., 2016; Rhoades et al., 2012), therefore it would be especially helpful to understand how models of high-quality social relationships impact future social bonds formed by offspring.

Interestingly, females that locomoted more during juvenile testing also changed zones more frequently during the present test, suggesting this behavior may be somewhat stable across time for an individual. Increased locomotion and zone changing may indicate a heightened state of arousal as well as greater separation distress. Therefore, females that exhibit more distress upon separation as juveniles may also exhibit greater anxiety and distress when facing social challenges as adults. Locomotion duration as infants and juveniles did not significantly explain

variability with regards to who females spent time with during preference testing, but infant separation distress was a significant predictor of other arousal behaviors (e.g., chest rubbing, long calling) and affiliative behaviors (lip-smacking). For example, females that exhibited greater vocalizations during IOF testing lip-smacked at their partners more during the 6-month post-paring test but less during the 1-week post-pairing test, and back-arch/tail-lashed (arch/lash) more during both of these tests. Similarly, females that locomoted more during infant testing arch/lashed more during both of these tests, and long called with their father more during the 1-week post-pairing test. Therefore, greater infant separation distress behavior is related to greater social interactions post-pairing. Our measures of infant and juvenile proximity maintenance also explained variability in arousal behaviors. In general, females that spend more time in proximity to their father as infants and juveniles arch/lash, chest rub, and long called with their father more post-pairing. Three of these behaviors (lip-smacking, arching/lashing, and long calling) are typically done in response to another conspecific, and are oftentimes done in coordination with the other individual. Long calling, for example, was scored only when the female was joining her father when he was long calling. While we did not score the behaviors of the father and the partner, another study in titi monkeys showed high coordination of behaviors such as lip-smacking and arching/lashing between bonded pair mates (Mercier et al., 2020). It is likely that females were coordinating their displays of affiliation and arousal with their fathers and partners during testing, which may indicate a higher-quality relationship between the subject and the stimulus animal. Future studies should investigate how the stimulus animals are behaving during preference testing and measure degrees of coordination.

Overall, the quality of relationship a daughter has to her father appears to set the foundation for the formation of a high-quality pair bond with her partner. Females exhibit

increased sociality when they have higher-quality relationships, and we found evidence to suggest that females may be able to somewhat maintain multiple selective attachments after establishing a pair bond if they have a high-quality relationship with their father as infants and juveniles. Juvenile predictors of relationship quality were particularly important predictors of female proximity maintenance, affiliation, and arousal, suggesting the quality of a relationship immediately preceding a new relationship may be more influential than infant relationship quality. Given that behavioral expressions of an attachment relationship are very similar between father-daughter bonds and adult pair bonds, and that we observed the potential for females to maintain bonds with their fathers after the formation of a pair bond with a conspecific, it is likely that the mechanisms driving females' attachment to their fathers and partners are overlapping. To test this prediction, we conducted a neuroimaging study to investigate the neural correlates of filial bonds and adult pair bonds.

Experiment 2

We measured brain glucose uptake using FDG PET scans while females were in their natal groups and 6-months post-pairing. FDG has been used as an approximation of brain activity in previous titi monkey studies (Arias del Razo et al., 2020, 2022; Bales et al., 2007; Hinde et al., 2016). At the beginning and end of Experiment 1, females underwent Experiment 2. Females were first scanned while still in their natal group to examine brain activity when they were tested with their fathers and alone. Females were later scanned at the completion of Experiment 1 when they were living with their pair bonded partner. Females were scanned with their partner and alone to identify overlapping brain activity underlying female attachment to the father and the partner. The present study is the first to directly compare neural correlates of filial and adult attachment in female titi monkeys.

We had predicted that overall glucose uptake would be enhanced at our post-pairing time point, as this was found in studies on male titi monkeys (Bales et al., 2007; Maninger et al., 2017). Our results did not support this prediction, with whole brain glucose uptake and uptake in our individual regions of interest being significantly lower post-pairing. A recent study by Arias del Razo and colleagues (Arias del Razo et al., 2022) found that females did not exhibit differences in glucose uptake post-pairing; however, females had higher glucose metabolism overall compared to males. Because our scans occurred two months later into pairing than the scans conducted in males, it is unclear whether females have a different response to pairing, or whether age more significantly impacted glucose uptake. Human research has demonstrated a decline in glucose metabolism with age (Martin, Friston, Colebach, & Frackowiak, 1991); therefore, it is difficult to disentangle the effects of pairing from the effects of age on this change in glucose metabolism. Future longitudinal studies should investigate changes in glucose metabolism across development in titi monkeys in conjunction with changes in glucose uptake following the formation of a pair to better understand interactions between pairing status and age.

Despite this overall pattern of decreased glucose metabolism in females 6-months post-pairing, we observed a fair amount of variability in glucose metabolism between subjects. While we had initially predicted that glucose uptake would decrease in all of our regions of interest in response to social separation, we did not find evidence to support that separation on its own elicited a significant change in glucose metabolism between scans when females were with their attachment figure (father or partner) and scans when they were separated from their current attachment figure. However, interactions between testing condition and quality of daughters'

relationships with their fathers did elucidate subtle differences in both whole brain glucose metabolism and activity in our regions of interest.

With regards to whole brain activity, measures of juvenile proximity maintenance predicted lower glucose metabolism when separated from their fathers and during both post-pairing scans. We then examined changes in the social salience network (SSN; comprised of the amygdala, hypothalamus, LS, NAc, VP, and VTA) and found this same exact interaction between juvenile relationship quality and testing condition. Females that spend more time in proximity to their fathers as juveniles exhibit lower SSN activity when separated from their father and when they are tested with their partner (both during the partner and separation conditions). This effect was moderate ($f^2 = 0.228$), suggesting the quality of a female's relationship with her father significantly predicts differences in neural responses to separations from the father and presence of the partner. It is hypothesized that lower brain metabolism in the face of a challenge may be indicative of better social support in humans (for a review, see (Coan, 2010). It is possible that we are observing similar patterns in titi monkey females with higher-quality relationships with their fathers, where they have relatively lower glucose metabolism when temporarily separated from their father. This may be due to the lingering stress buffering effects that fathers may have on their daughters. These same females with higher-quality relationships with their fathers may similarly be better buffered by their partners post-pairing, resulting in a further reduction of whole brain glucose metabolism and activity in the SSN.

Interestingly, *Parent Affiliation* had the opposite effect when females were tested post-pairing, both in the partner and separation conditions, where females exhibited an increase in SSN activity. While human studies have demonstrated that parents' relationship quality can impact their children's future relationship quality with partners as adults (Gager et al., 2016;

Rhoades et al., 2012), the neural mechanism underlying this relationship are still unclear. The present study is the first to examine this relationship in a non-human primate (NHP) model, so our interpretations of this finding are currently speculative. It is possible that observation of higher-quality relationships may differently activate socially salient brain regions to make females more responsive to changes in their social relationships, like the formation of a pair bond and separation from a bonded partner. Overall differences in activity in brain regions is difficult to fully interpret solely using measures of glucose metabolism. It is unclear whether these changes represent increases in glutamatergic or GABA-ergic activity. Knowing whether a brain region exhibiting changes in activity is being inhibited or excited may aid in interpretations.

Two additional brain regions that were examined in the present study due to their interactions with the SSN were the cerebellum and the periaqueductal gray (PAG). Juveniles that spend a greater amount of time in proximity to their fathers also exhibit lower cerebellum and PAG activity specifically when they are separated from their fathers. These findings mirror our findings in the whole brain and the SSN. Higher *Parent Affiliation* scores were positively correlated with PAG activity during both post-pairing scans, just like we observed with the SSN. Therefore, observation of high-quality parent relationships may similarly impact the PAG to make them more responsive to changes in social relationships.

Unexpectedly, measures of infant proximity maintenance and juvenile separation distress resulted in opposite patterns of PAG activation. Females that spent a greater amount of time touching the grate during IOF testing exhibited reduced activity in the PAG when with their partners, but enhanced PAG activity when separated from them. Conversely, females that vocalize more when separated from their fathers as juveniles (*Juvenile Vocalization*) exhibit increased PAG activity when with their partners and reduced PAG activity when separated

specifically from their partners. Interestingly, *Juvenile Vocalization* was positively correlated with PAG activity when females were separated from their fathers. The PAG has been identified as playing an important role in guinea pig separation distress and human sadness (Panksepp, 2003). This may explain why certain measures of relationship quality (*IOF Grate*, *Juvenile Vocalization*) predict greater PAG activity during separation conditions, but this differed depending on who the current attachment figure was. It was only during post-pairing tests when *Juvenile Vocalization* correlated in the opposite directions with PAG activity. *Juvenile Vocalization* was our only measure of separation distress that significantly explained neural activity. All other measures that explained variability in glucose metabolism were related to measures of proximity maintenance and affiliation between parents. Therefore, separation distress may play a different functional role in relationship quality that is associated with neural activity that differs from proximity maintenance. Heightened PAG activity when with the partner and reduced PAG activity when separated from the partner may reflect a different functional role for the PAG that may be heightened in females that exhibit greater distress upon separation from their fathers. For example, in birds, the midbrain central gray (which we are referring to as the PAG in the present study on titi monkeys) is involved in the mesolimbic dopamine pathway and activation of neurons in this brain region is associated with the expression of courtship behaviors between partners (Goodson, Kabelik, Kelly, Rinaldi, & Klatt, 2009). It is possible that females that exhibit greater distress upon separation from their fathers have altered neural circuitry that makes them more sensitive to dopaminergic activation of the PAG when they are with their partners. Conversely, females that exhibit greater proximity maintenance with their father may have altered neural circuitry that makes them more sensitive to opioid activity in the PAG. The hypothalamus also has oxytocinergic and vasopressin-ergic projections to the PAG that regulate

social pain and rejection pathways (Jiang, Bao, Sun, Liu, & Yang, 2019; Yang et al., 2007), so differences in relationship quality may also differently impact these neural connections. We are unable to identify which neuropeptide systems were activated during our testing conditions; therefore, future studies should specifically examine differences in OT, AVP, dopamine, and opioid system activations to better disentangle the impact of relationship quality on neural activity.

Overall, we found that females exhibited reduced glucose metabolism post-pairing; however, differences in daughters' quality of relationship with their fathers explained variability in glucose uptake across our regions of interest. In general, measures of female proximity maintenance predicted increased neural activity during separation conditions and reduced neural activity when females were tested with their current attachment figure. In contrast, measures of separation distress resulted in lowered activity during social separations, specifically in the PAG, highlighting the need for investigating differences in OT, AVP, dopamine, and opioid activity across our regions of interest. These findings emphasize the need for incorporating measures of relationship quality when investigating the neural correlates of social bonds.

Conclusions. Our main objectives were to 1) identify foundational neurobiological and behavioral mechanisms underlying female attachment relationships and 2) determine how relationship quality affects bonding mechanisms. Across our two experiments, we found evidence to suggest that measures of relationship quality significantly explain variability in behavioral and neural correlates of social bonds in female titi monkeys. In particular, higher-quality relationships with the father established a stronger foundation for forming and maintaining an adult pair bond. We also found evidence to suggest that females are capable of maintaining a relationship with their father least 6-months post pairing, as evidenced by a desire

to maintain proximity to the father if they exhibit higher-quality relationships with their fathers. Our examination of neural activity pre- and post-pairing suggest a high degree of overlap between circuitry driving filial attachment and adult pair bonding, as patterns of activity were similar across the two time points, despite overall activity being lower post-pairing. It is likely that differences in OT, AVP, dopamine, and opioid activity underly the differences observed in neural activity based on daughter's relationship with their fathers. Findings from the present studies advance current knowledge of the neurobiological mechanisms foundational to attachment relationships, and help inform how social disruptions caused by events such as the global pandemic may differently impact humans based on the quality of their social bonds.

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TABLES

Table 1. Ethogram for partner-father preference test outcome variables

Behavior	Definition
Zone Ratio	Ratio indicating which zone (partner, father, other) subject spends the most time in. Calculated by multiplying time in partner zone by +1, time in father zone by -1, time in other zone by 0, and summing those three values
Percent Social	Percentage of total test time subject is located in either the partner or the father zone. Calculated by adding time in partner zone and time in father zone, dividing by the total time, and multiplying by 100.
Partner Zone	Total time subject is located in the preference zone next to the partner
Father Zone	Total time subject is located in the preference zone next to the father pair
Other Zone	Total time subject is located in any location in the test cage other than the partner or father preference zones
Partner Grate	Total time subject touches the grated window between the test cage and their partner's cage using hands or feet
Father Grate	Total time subject touches the grated window between the test cage and the father's cage using hands or feet
Zone Change	Total number of times subject changes between partner, father, and other zones during testing
Partner-directed Lip-smack	Subject makes rapid lip movement accompanied by smacking sound while facing partner
Father-directed Lip-smack	Subject makes rapid lip movement accompanied by smacking sound while facing father

Back-arch/Tail-lash	Subject raises dorsal surface of the back in an arched position (sometimes to the point where the front hands come off of the perch, but not limited to this) AND/OR laterally whips the tail (a bout rather than individual lashes).
Chest Rub	Subject displays rubbing of the chest in a variety of forms. This may include rubbing the chest with the hand or rubbing the chest directly onto a perch or wall. The display often includes sniffing of the surface where the chest was rubbed.
Partner Long call	Subject begins long calling within 3 seconds of the partner calling. Test animal must be joining the song of the partner.
Father Long call	Subject begins long calling within 3 seconds of the father calling. Test animal must be joining the song of the father.
Partner-directed Aggression	Subject displays anything that resembles aggression at the partner's grate. The subject must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.
Father-directed Aggression	Subject displays anything that resembles aggression at the father's grate. The subject must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.
Receive Aggression from Partner	Partner displays anything that resembles aggression while subject is in the partner zone. The partner must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.

Receive Aggression from Father	Father displays anything that resembles aggression while subject is in the father zone. The father must display at least 2 out of the following 4 behaviors (unless there is clear aggression from the stimulus animal—then only 1 of the following 4 behaviors is necessary): 1) swiftly approaching the grate, 2) jumping/pouncing on the grate, 3) repeated fast touching of the grate, or 4) grabbing and pulling on the grate.

Table 2. Ethogram for relationship quality predictors

Behavior	Definition
Separation Distress Behaviors	
IOF Locomotion	Percent change in line crossing from the father to the empty box condition of infant open field (IOF) testing
IOF Vocalization	Percent change in vocalizations emitted from the father to the empty box condition of IOF testing
Juvenile Locomotion	Percent change in locomotion behavior from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Juvenile Vocalization	Percent change in total vocalizations emitted from the stress buffered to the separation distress condition of juvenile separation testing when subjects are treated with saline
Proximity Maintenance Behaviors	
Juvenile Proximity	Percentage of time subject spends in proximity, contact, or tail-twining with the father during a 15-minute reunion period immediately following juvenile social separation testing when subjects are treated with saline
Juvenile Parent Preference	Percentage of time subject spends in the parent proximity zone of juvenile parent preference tests when subjects are treated with saline
Juvenile Parent Choice	Percentage of times subject chooses parents during the catch and release testing immediately following juvenile parent preference tests when subjects are treated with saline
IOF Proximity	Percentage of time subject spends in proximity to the father during the father condition of IOF testing
IOF Grate	Percentage of time subject spends touching the grate in the IOF arena during the father condition of IOF testing
Infant Proximity	Percentage of time subject was carried by the father during the first nine months of life as measured during daily scan samples
Affiliation Behaviors	

Pair Affiliation	Percentage of time subject spent in proximity (within ~6 inches of one another), contact (physical body contact), or tail-twining (sitting with tails wrapped together) with their partner during the first six months of pairing as measured during daily scan samples
Parent Affiliation	Percentage of time parents of subjects spent in proximity (within ~6 inches of one another), contact (physical body contact), or tail-twining (sitting with tails wrapped together) with each other during the first 14 months of the subject's life as measured during daily scan samples

Table 3. Pearson correlation matrix of relationship quality variables. Significant correlations ($p < .05$) indicated in bold. IOFLine = *IOF Locomotion;* IOFVoc = *IOF Vocalization;* JuvVoc = *Juvenile Vocalization;* JuvLoc = *Juvenile Locomotion;* JuvReun = *Juvenile Proximity;* PropPar = *Juvenile Preference;* CnRPar = *Juvenile Choice;* InfCarry = *Infant Proximity;* IOFProx = *IOF Proximity;* IOFGrate = *IOF Grate;* ParAff = *Parent Affiliation;* PairAff = *Pair Affiliation*

	IOFLine	IOFVoc	JuvVoc	JuvLoc	JuvReun	PropPar	CnRPar	InfCarry	IOFProx	IOFGrate	ParAff	PairAff
IOFLine	1.00											
IOFVoc	**0.69**	1.00										
JuvVoc	**0.52**	**0.42**	1.00									
JuvLoc	**-0.20**	**-0.25**	**-0.35**	1.00								
JuvReun	**-0.49**	**-0.44**	**-0.62**	**0.20**	1.00							
PropPar	0.10	**0.44**	**-0.37**	**0.23**	0.09	1.00						
CnRPar	**0.27**	**0.20**	**0.29**	**0.40**	**-0.34**	**0.44**	1.00					
InfCarry	0.00	**0.56**	-0.13	0.14	-0.01	**0.41**	**-0.19**	1.00				
IOFProx	**0.49**	0.11	-0.05	**-0.22**	**-0.26**	**0.31**	0.07	**-0.22**	1.00			
IOFGrate	0.13	**-0.19**	**-0.36**	-0.11	**0.25**	-0.06	**-0.36**	**-0.46**	**0.42**	1.00		
ParAff	**-0.52**	**-0.34**	**-0.26**	**-0.31**	0.02	-0.01	**-0.18**	0.09	0.04	**-0.44**	1.00	
PairAff	0.08	0.09	0.15	**-0.47**	**0.49**	**-0.38**	**-0.47**	-0.05	**-0.38**	0.14	**-0.18**	1.00

Table 4. Results from Experiment 1 general linear mixed-effects models and zero-inflated mixed effects models.

4a. Zone Ratio

	β	SE	t-value	p
(Intercept)	-270.422	84.793	-3.189	0.002
TestNo2	59.511	119.916	0.496	0.621
TestNo3	328.889	119.916	2.743	0.007
PropPar	-7.780	2.278	-3.415	< .001

4b. Percent Social

	β	SE	t-value	p
(Intercept)	66.928	1.976	33.875	< .001
TestNo2	1.481	2.794	0.530	0.597
TestNo3	7.708	2.794	2.759	0.007
PropPar	0.441	0.233	1.896	0.061
CnRPar	-0.110	0.242	-0.457	0.649
InfCarry	0.253	1.076	0.235	0.815
IOFGrate	-0.056	0.274	-0.203	0.839
IOFLine	-0.020	0.025	-0.787	0.433
IOFVoc	-0.191	0.096	-1.983	0.050
JuvVoc	0.238	0.084	2.847	0.005
TestNo2:PropPar	0.025	0.329	0.076	0.940
TestNo3:PropPar	-0.265	0.329	-0.806	0.422
TestNo2:CnRPar	-0.299	0.342	-0.874	0.384
TestNo3:CnRPar	-0.064	0.342	-0.187	0.852
TestNo2:InfCarry	-3.030	1.522	-1.992	0.049
TestNo3:InfCarry	-0.567	1.522	-0.372	0.710
TestNo2:IOFGrate	-0.464	0.388	-1.196	0.234
TestNo3:IOFGrate	0.151	0.388	0.388	0.699
TestNo2:IOFLine	-0.023	0.036	-0.652	0.516
TestNo3:IOFLine	0.002	0.036	0.047	0.963
TestNo2:IOFVoc	0.197	0.136	1.452	0.149
TestNo3:IOFVoc	0.147	0.136	1.080	0.283
TestNo2:JuvVoc	-0.091	0.118	-0.771	0.442
TestNo3:JuvVoc	-0.204	0.118	-1.721	0.088

4c. Partner Zone

	β	SE	t-value	p
(Intercept)	20.552	0.960	21.412	< .001

TestNo2	0.521	1.357	0.384	0.702
TestNo3	5.196	1.357	3.828	< .001
JuvReun	0.060	0.021	2.853	0.005
PropPar	-0.155	0.030	-5.167	< .001
CnRPar	0.101	0.036	2.806	0.006

4d. Father Zone

	β	SE	t-value	p
(Intercept)	28.091	1.317	21.323	<2e-16
ObsNo	-0.811	0.373	-2.173	0.032
PropPar	0.055	0.032	1.710	0.131

4e. Other Zone

	β	SE	t-value	p
(Intercept)	23.670	0.837	28.268	<2e-16
TestNo2	-0.907	1.184	-0.766	0.445
TestNo3	-2.952	1.184	-2.493	0.014
PropPar	-0.101	0.044	-2.319	0.022
InfCarry	0.082	0.229	0.360	0.720
IOFGrate	0.008	0.051	0.160	0.873
IOFLine	0.010	0.006	1.571	0.119
IOFVoc	0.040	0.027	1.474	0.143
JuvVoc	-0.047	0.020	-2.281	0.024
JuvLoc	0.003	0.002	1.749	0.083

4f. Partner Grate Touch

	β	SE	t-value	p
(Intercept)	4.058	0.192	21.178	< .001
TestNo2	-0.280	0.232	-1.208	0.229
TestNo3	0.120	0.232	0.515	0.607
JuvReun	0.019	0.010	1.823	0.166
PropPar	-0.012	0.011	-1.157	0.285
IOFLine	-0.001	0.001	-1.085	0.357
JuvVoc	0.006	0.004	1.482	0.235
PairAff	-0.034	0.025	-1.338	0.273
TestNo2:PropPar	-0.037	0.011	-3.401	< .001
TestNo3:PropPar	0.014	0.011	1.289	0.200

4g. Father Grate Touch

	β	SE	t-value	p

(Intercept)	5.554	0.301	18.431	< .001
TestNo2	-0.271	0.223	-1.213	0.228
TestNo3	-0.435	0.223	-1.949	0.054
ObsNo	-0.260	0.064	-4.043	< .001
InfCarry	0.112	0.070	1.587	0.253
IOFProx	0.005	0.011	0.494	0.650
IOFVoc	-0.008	0.007	-1.163	0.346
JuvVoc	0.004	0.004	0.942	0.446
JuvLoc	-0.001	0.001	-1.509	0.270
ParAff	-0.067	0.044	-1.501	0.231
TestNo2:IOFProx	-0.006	0.009	-0.695	0.489
TestNo3:IOFProx	0.030	0.009	3.184	0.002
TestNo2:IOFVoc	0.008	0.004	2.156	0.033
TestNo3:IOFVoc	-0.003	0.004	-0.692	0.490
TestNo2:ParAff	0.039	0.033	1.182	0.240
TestNo3:ParAff	-0.022	0.033	-0.683	0.496

4h. Zone Change Frequency

	β	SE	t-value	p
(Intercept)	4.733	0.093	50.892	< .001
TestNo2	-0.484	0.131	-3.700	< .001
TestNo3	0.010	0.131	0.078	0.938
CnRPar	-0.014	0.003	-4.431	0.007
JuvLoc	0.001	0.000	3.791	0.013
ParAff	-0.041	0.008	-5.165	0.004

4i. Partner-directed Lip-smacking

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	1.625	0.247	6.582	< .001
TestNo2	0.652	0.213	3.058	0.002
TestNo3	0.504	0.218	2.313	0.021
ObsNo	-0.289	0.034	-8.591	< .001
IOFLine	-0.005	0.001	-3.688	< .001
IOFVoc	-0.009	0.005	-1.859	0.063
TestNo2:IOFVoc	0.011	0.004	2.654	0.008
TestNo3:IOFVoc	0.025	0.004	5.884	< .001
Zero-part coefficients				
(Intercept)	-0.510	0.384	-1.328	0.184
TestNo2	-1.806	0.717	-2.520	0.012

TestNo3	-1.471	0.743	-1.979	0.048

4j. Father-directed Lip-smacking

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	-6.774	19.722	-0.344	0.731
TestNo2	8.549	19.723	0.433	0.665
TestNo3	9.557	19.721	0.485	0.628
ObsNo	-0.527	0.041	-12.778	< .001
PropPar	-0.047	0.137	-0.345	0.730
IOFProx	-0.424	0.892	-0.475	0.635
PairAff	-0.521	1.167	-0.446	0.656
TestNo2:PropPar	0.095	0.137	0.693	0.488
TestNo3:PropPar	0.072	0.137	0.525	0.600
TestNo2:IOFProx	0.392	0.892	0.439	0.661
TestNo3:IOFProx	0.435	0.892	0.488	0.626
TestNo2:PairAff	0.535	1.168	0.458	0.647
TestNo3:PairAff	0.572	1.168	0.490	0.624
Zero-part coefficients				
(Intercept)	-2.664	9.262	-0.288	0.774
TestNo2	-6.634	6.773	-0.979	0.327
TestNo3	-6.005	7.096	-0.846	0.397

4k. Back-arching/Tail-lashing

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	2.499	0.182	13.730	< .001
TestNo2	0.575	0.161	3.579	< .001
TestNo3	0.950	0.146	6.520	< .001
ObsNo	-0.472	0.024	-19.462	< .001
JuvReun	0.038	0.009	4.168	< .001
PropPar	-0.076	0.015	-5.253	< .001
CnRPar	0.053	0.014	3.712	< .001
IOFLine	-0.009	0.002	-4.256	< .001
IOFVoc	0.025	0.004	5.981	< .001
TestNo2:JuvReun	0.000	0.008	0.041	0.967
TestNo3:JuvReun	-0.020	0.007	-2.752	0.006
TestNo2:PropPar	0.053	0.012	4.474	< .001
TestNo3:PropPar	0.047	0.012	3.816	< .001

TestNo2:CnRPar	-0.026	0.013	-2.025	0.043
TestNo3:CnRPar	-0.049	0.012	-3.956	< .001
TestNo2:IOFLine	0.007	0.002	3.927	< .001
TestNo3:IOFLine	0.009	0.002	5.495	< .001
TestNo2:IOFVoc	-0.010	0.003	-3.434	< .001
TestNo3:IOFVoc	-0.008	0.003	-2.646	0.008
Zero-part coefficients				
(Intercept)	-1.279	0.776	-1.648	0.099
ObsNo	0.553	0.207	2.668	0.008
PartnerEXPnaïve	-5.422	1.712	-3.167	0.002
JuvReun	-0.072	0.020	-3.615	< .001
PropPar	0.096	0.033	2.930	0.003
IOFVoc	-0.049	0.014	-3.607	< .001

4l. Chest Rub

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	1.445	0.387	3.732	< .001
TestNo2	-0.895	0.532	-1.685	0.092
TestNo3	0.664	0.361	1.838	0.066
ObsNo	-0.139	0.046	-3.050	0.002
IOFGrate	-0.056	0.019	-2.971	0.003
PairAff	-0.011	0.032	-0.346	0.729
TestNo2:IOFGrate	0.062	0.020	3.094	0.002
TestNo3:IOFGrate	0.034	0.020	1.669	0.095
TestNo2:PairAff	0.135	0.044	3.045	0.002
TestNo3:PairAff	0.032	0.032	0.978	0.328
Zero-part coefficients				
(Intercept)	1.870	0.619	3.023	0.003
TestNo2	-0.919	0.764	-1.203	0.229
TestNo3	-2.272	0.790	-2.877	0.004
IOFGrate	-0.023	0.038	-0.596	0.551
PairAff	-0.220	0.045	-4.858	< .001
TestNo2:IOFGrate	0.040	0.047	0.849	0.396
TestNo3:IOFGrate	0.108	0.049	2.218	0.027

4m. Long call with Father

	β	SE	Z-value	p
Response-part coefficients				
(Intercept)	-0.070	0.469	-0.149	0.882

TestNo2	-0.529	0.436	-1.213	0.225
TestNo3	0.646	0.286	2.260	0.024
ObsNo	-0.034	0.081	-0.414	0.679
IOFGrate	-0.007	0.022	-0.322	0.748
IOFLine	0.000	0.003	-0.161	0.872
TestNo2:IOFGrate	-0.068	0.028	-2.445	0.014
TestNo3:IOFGrate	-0.001	0.016	-0.051	0.959
TestNo2:IOFLine	0.005	0.002	3.046	0.002
TestNo3:IOFLine	0.002	0.002	1.066	0.286
Zero-part coefficients				
(Intercept)	-1.667	0.752	-2.217	0.027
ObsNo	0.519	0.213	2.434	0.015
IOFLine	-0.004	0.002	-2.204	0.028

Table 5. Results from Experiment 2 general linear mixed-effects models and zero-inflated mixed effects models.

5a. Social Salience Network

	β	SE	t-value	p
(Intercept)	7.534	0.054	138.837	< .001
Conditionpartner	-0.365	0.034	-10.855	< .001
ConditionsepF	-0.074	0.034	-2.210	0.028
ConditionsepP	-0.421	0.034	-12.494	< .001
RegionHYP	-1.689	0.041	-40.972	< .001
RegionLS	-2.998	0.041	-72.710	< .001
RegionNAC	-0.981	0.041	-23.783	< .001
RegionVP	-4.025	0.041	-97.634	< .001
RegionVTA	-3.268	0.041	-79.252	< .001
JuvReun	0.000	0.002	-0.271	0.793
ParAff	0.009	0.006	1.382	0.200
Conditionpartner:JuvReun	-0.006	0.001	-4.854	< .001
ConditionsepF:JuvReun	-0.012	0.001	-10.218	< .001
ConditionsepP:JuvReun	-0.002	0.001	-1.537	0.125
Conditionpartner:ParAff	0.022	0.005	4.787	< .001
ConditionsepF:ParAff	-0.007	0.005	-1.501	0.134
ConditionsepP:ParAff	0.017	0.005	3.718	< .001

5b. Whole Brain

	β	SE	t-value	p
(Intercept)	12.633	0.054	235.496	< .001
Conditionpartner	-0.378	0.074	-5.098	< .001
ConditionsepF	-0.050	0.074	-0.676	0.502
ConditionsepP	-0.411	0.074	-5.540	< .001
JuvReun	-0.003	0.002	-1.385	0.176
CnRPar	-0.002	0.003	-0.523	0.604
IOFProx	0.005	0.003	1.916	0.064
IOFLine	-0.001	0.000	-1.244	0.223
Conditionpartner:JuvReun	-0.007	0.003	-2.354	0.023
ConditionsepF:JuvReun	-0.014	0.003	-4.484	< .001
ConditionsepP:JuvReun	-0.002	0.003	-0.786	0.436
Conditionpartner:CnRPar	0.001	0.004	0.282	0.780
ConditionsepF:CnRPar	0.001	0.004	0.261	0.795
ConditionsepP:CnRPar	-0.010	0.004	-2.267	0.028
Conditionpartner:IOFProx	0.001	0.004	0.363	0.718

ConditionsepF:IOFProx	-0.004	0.004	-1.168	0.249
ConditionsepP:IOFProx	0.003	0.004	0.861	0.393
Conditionpartner:IOFLine	-0.001	0.001	-1.521	0.135
ConditionsepF:IOFLine	0.000	0.001	-0.181	0.857
ConditionsepP:IOFLine	0.000	0.001	0.414	0.681

5c. Cerebellum	β	SE	t-value	p
(Intercept)	10.705	0.091	117.090	< .001
Conditionpartner	-0.370	0.129	-2.858	0.008
ConditionsepF	-0.056	0.129	-0.429	0.671
ConditionsepP	-0.455	0.129	-3.520	0.002
JuvReun	-0.001	0.003	-0.333	0.742
ParAff	0.012	0.006	1.992	0.057
Conditionpartner:JuvReun	-0.005	0.004	-1.089	0.286
ConditionsepF:JuvReun	-0.012	0.004	-2.770	0.010
ConditionsepP:JuvReun	-0.001	0.004	-0.296	0.769

5d. Periaqueductal Gray (PAG)	β	SE	t-value	p
(Intercept)	5.491	0.056	97.325	< .001
Conditionpartner	-0.363	0.060	-6.071	< .001
ConditionsepF	-0.083	0.060	-1.382	0.173
ConditionsepP	-0.442	0.060	-7.403	< .001
JuvReun	-0.007	0.003	-2.560	0.051
CnRPar	-0.003	0.003	-1.119	0.379
IOFProx	0.004	0.002	1.558	0.259
IOFGrate	-0.016	0.006	-2.697	0.062
JuvVoc	-0.006	0.001	-3.985	0.008
ParAff	-0.023	0.012	-1.965	0.116
Conditionpartner:JuvReun	0.001	0.003	0.527	0.601
ConditionsepF:JuvReun	-0.009	0.003	-3.207	0.002
ConditionsepP:JuvReun	0.003	0.003	1.096	0.278
Conditionpartner:IOFGrate	0.010	0.005	2.090	0.042
ConditionsepF:IOFGrate	0.004	0.005	0.842	0.404
ConditionsepP:IOFGrate	0.023	0.005	4.757	< .001
Conditionpartner:JuvVoc	0.006	0.002	4.244	< .001
ConditionsepF:JuvVoc	0.003	0.002	2.203	0.032
ConditionsepP:JuvVoc	0.006	0.002	3.938	< .001
Conditionpartner:ParAff	0.044	0.011	4.082	< .001
ConditionsepF:ParAff	0.005	0.011	0.495	0.623
ConditionsepP:ParAff	0.054	0.011	5.058	< .001

Table 6. Results from Experiment 2 Tukey's post-hoc comparisons of relative brain region glucose uptake. HYP = Hypothalamus; AMY = Amygdala; LS = Lateral Septum; NAC = Nucleus Accumbens; VP = Ventral Pallidum; VTA = Ventral Tegmental Area.

	β	SE	Z value	p
HYP - AMY == 0	-1.689	0.041	-40.972	< .001
LS - AMY == 0	-2.998	0.041	-72.710	< .001
NAC - AMY == 0	-0.981	0.041	-23.783	< .001
VP - AMY == 0	-4.025	0.041	-97.634	< .001
VTA - AMY == 0	-3.268	0.041	-79.252	< .001
LS - HYP == 0	-1.309	0.041	-31.738	< .001
NAC - HYP == 0	0.709	0.041	17.189	< .001
VP - HYP == 0	-2.336	0.041	-56.662	< .001
VTA - HYP == 0	-1.578	0.041	-38.280	< .001
NAC - LS == 0	2.017	0.041	48.927	< .001
VP - LS == 0	-1.028	0.041	-24.924	< .001
VTA - LS == 0	-0.270	0.041	-6.542	< .001
VP - NAC == 0	-3.045	0.041	-73.851	< .001
VTA - NAC == 0	-2.287	0.041	-55.469	< .001
VTA - VP == 0	0.758	0.041	18.382	< .001

FIGURES

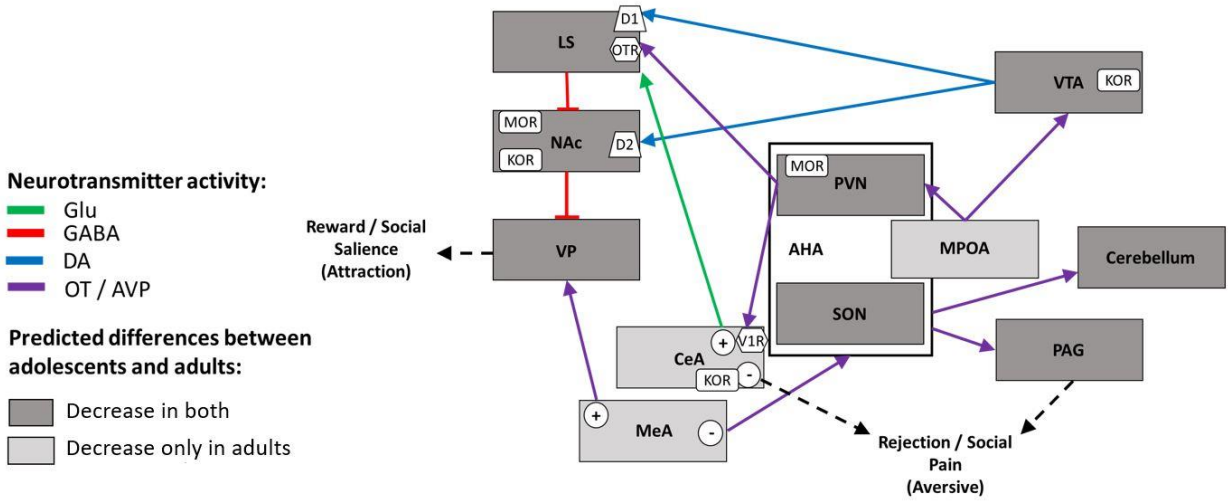


Figure 1. Predictions for Experiment 2 regarding brain regions involved in father-daughter bonds and adult pair-bonds. Abbreviations: → = excitatory input; –| = inhibitory input; Glu = glutamate; GABA = Gamma aminobutyric acid; DA = dopamine; OT = oxytocin; AVP = arginine-vasopressin; D1 = dopamine receptor 1; D2 = dopamine receptor 2; OTR = oxytocin receptor; MOR = mu opioid receptor; KOR = kappa opioid receptor; V1R = arginine-vasopressin receptor 1; LS = lateral septum; NAc = nucleus accumbens; VP = ventral pallidum; CeA = central amygdala; MeA = medial amygdala; PVN = paraventricular nucleus of hypothalamus; SON = supraoptic nucleus of hypothalamus; AHA = anterior hypothalamus; MPOA = medial preoptic area of hypothalamus; VTA = ventral tegmental area; PAG = periaqueductal gray

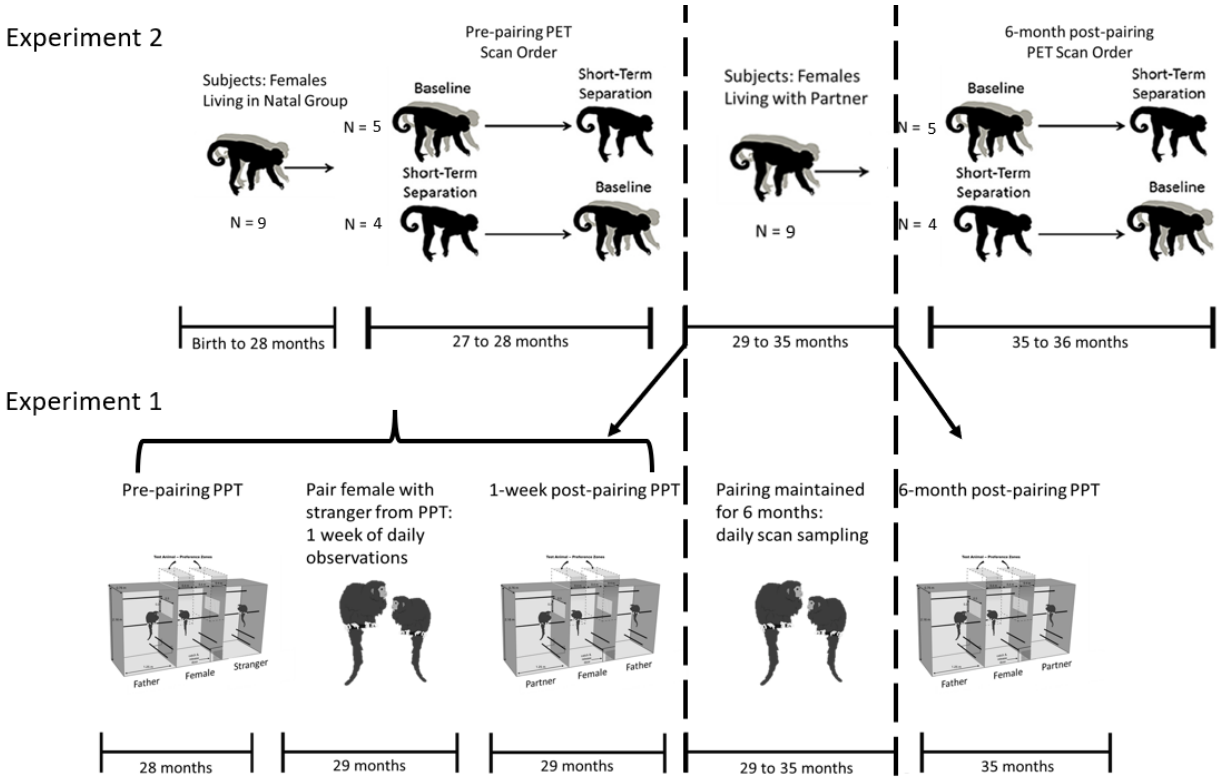


Figure 2. Timeline for Experiments 1 and 2. Age of subject indicated in months. PET = Positron Emission Tomography; PPT = Partner Preference Test

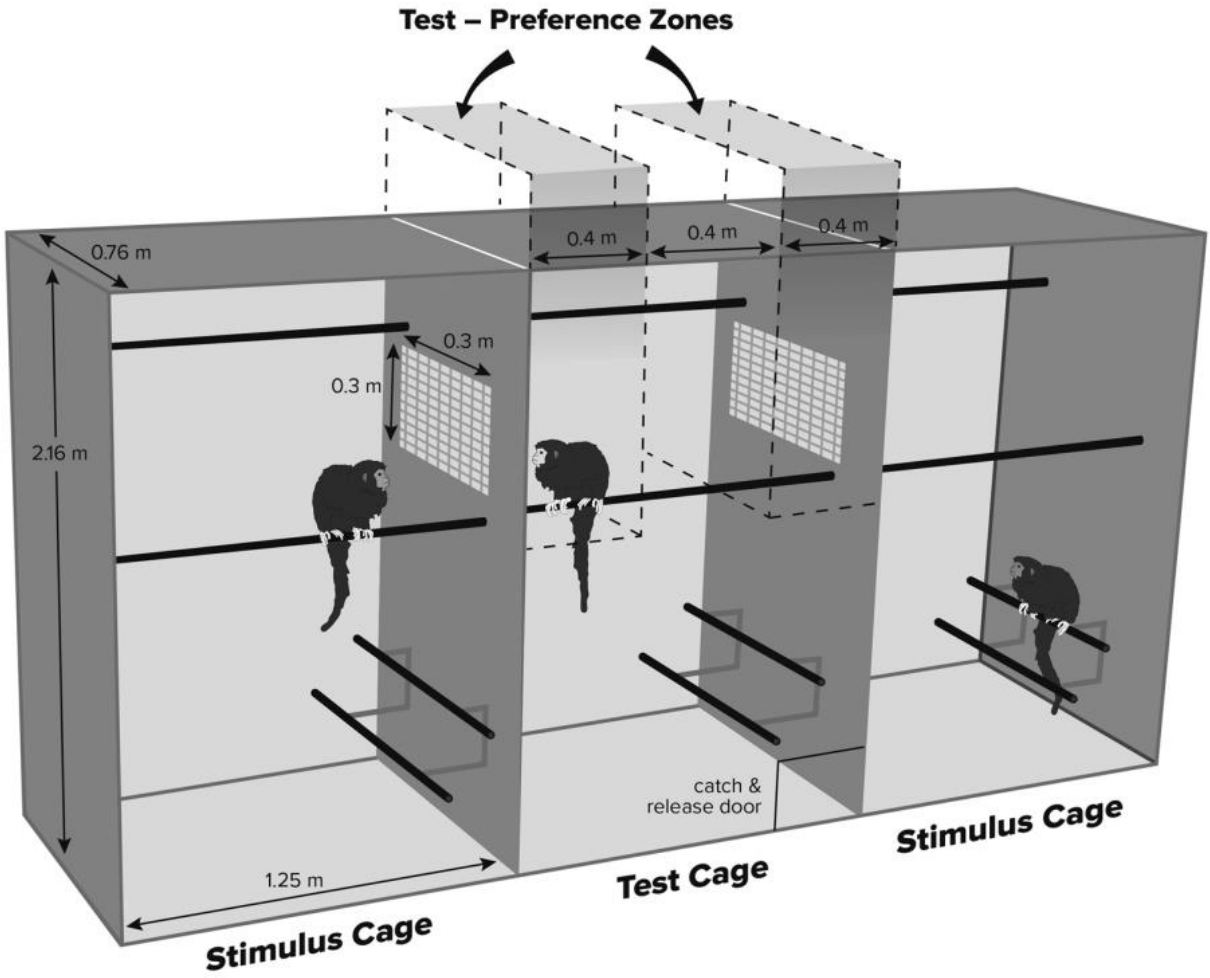


Figure 3. Schematic of Experiment 1 preference test arena (Rothwell et al., 2020).

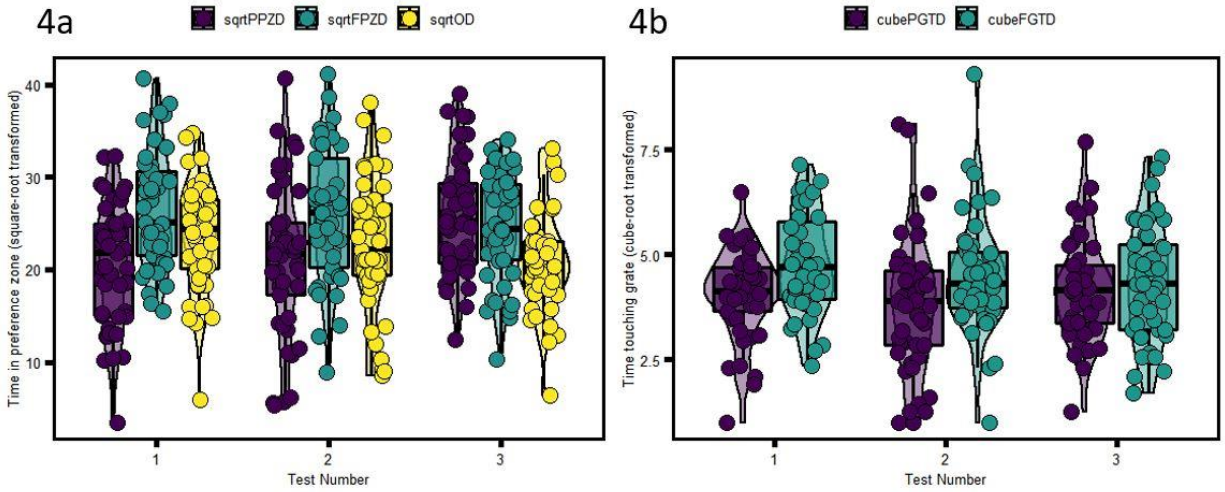


Figure 4. Summary of time females spend a) in preference zones and b) touching stimulus grates separated by *Test Number*. PPZD = Partner preference zone duration; FPZD = Father preference zone duration; OD = Other (or "non-social") zone duration; PGTD = Partner grate touch duration; FGTD = Father preference zone duration; Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

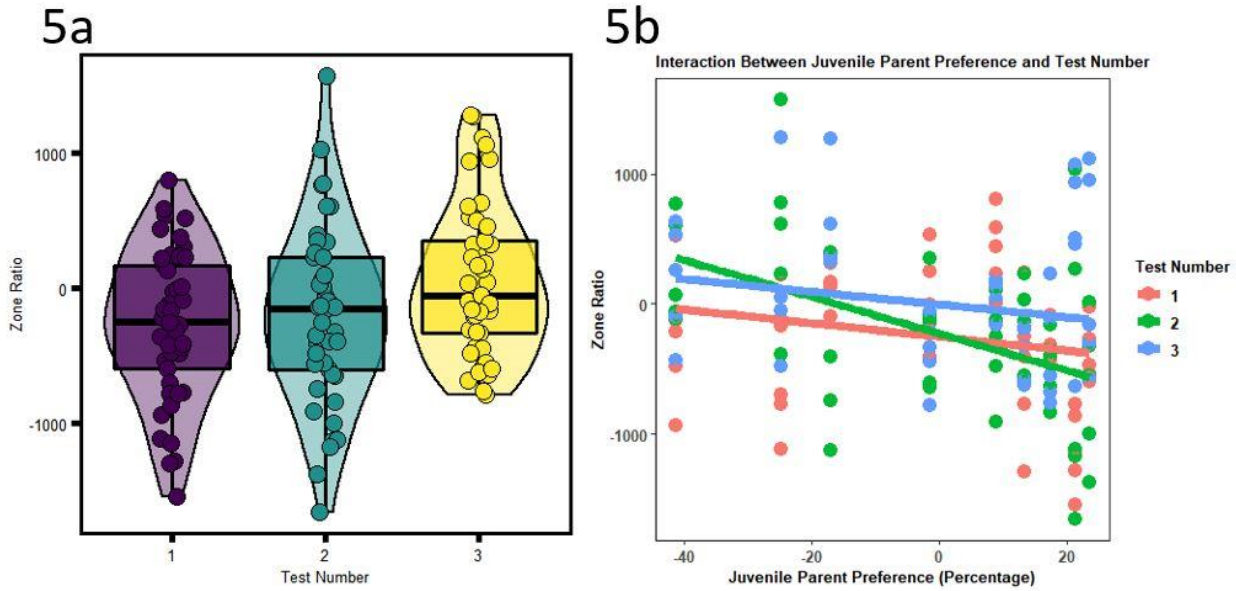


Figure 5. Zone Ratio score: a) the main effect of *Test Number* and interaction effects between *Test Number* and b) *Juvenile Parent Preference*. Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

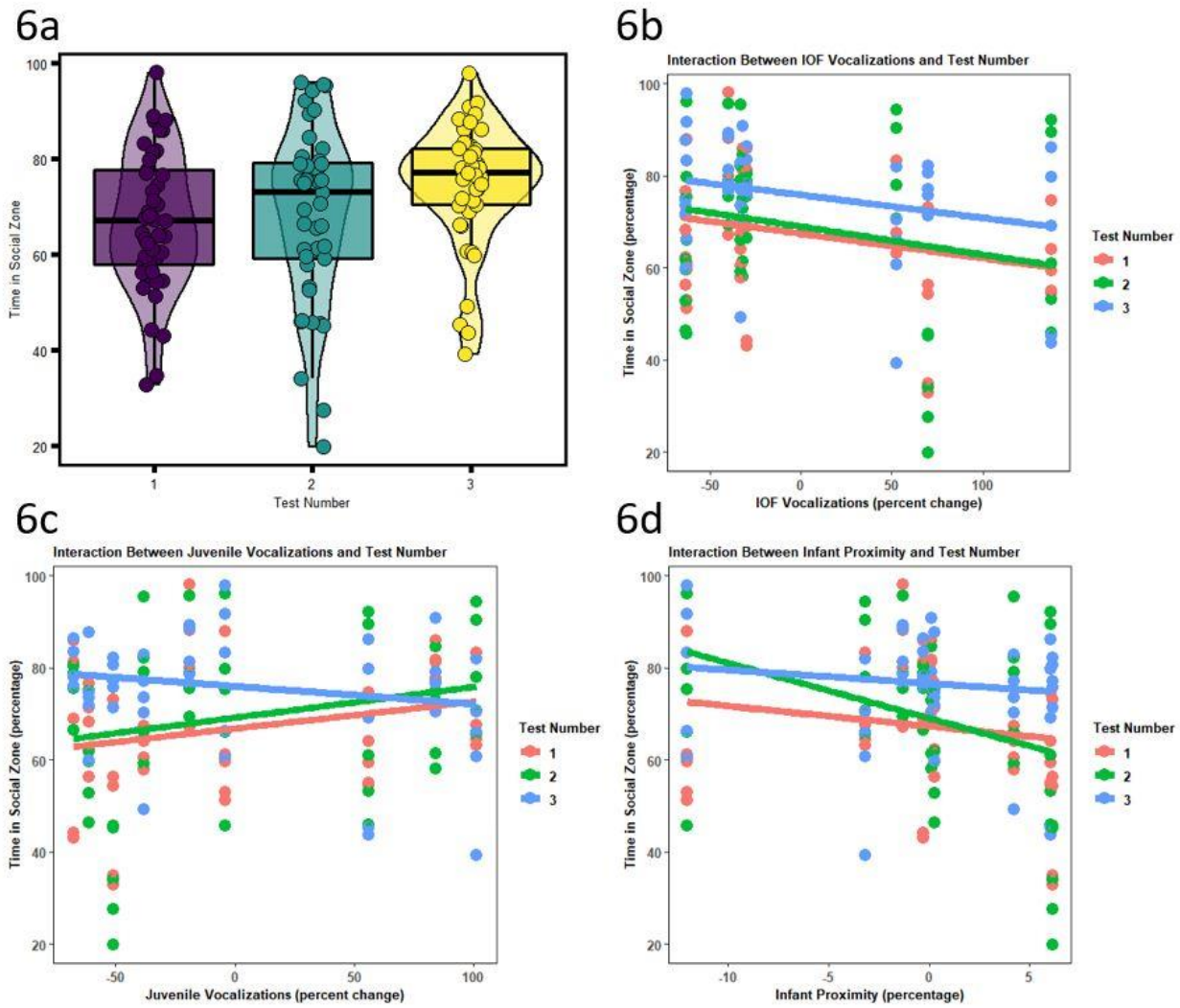


Figure 6. Percent Social score: a) the main effect of *Test Number*, interaction effects between *Test Number* and b) *IOF Vocalizations*, c) *Juvenile Vocalizations*, and d) *Infant Proximity.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

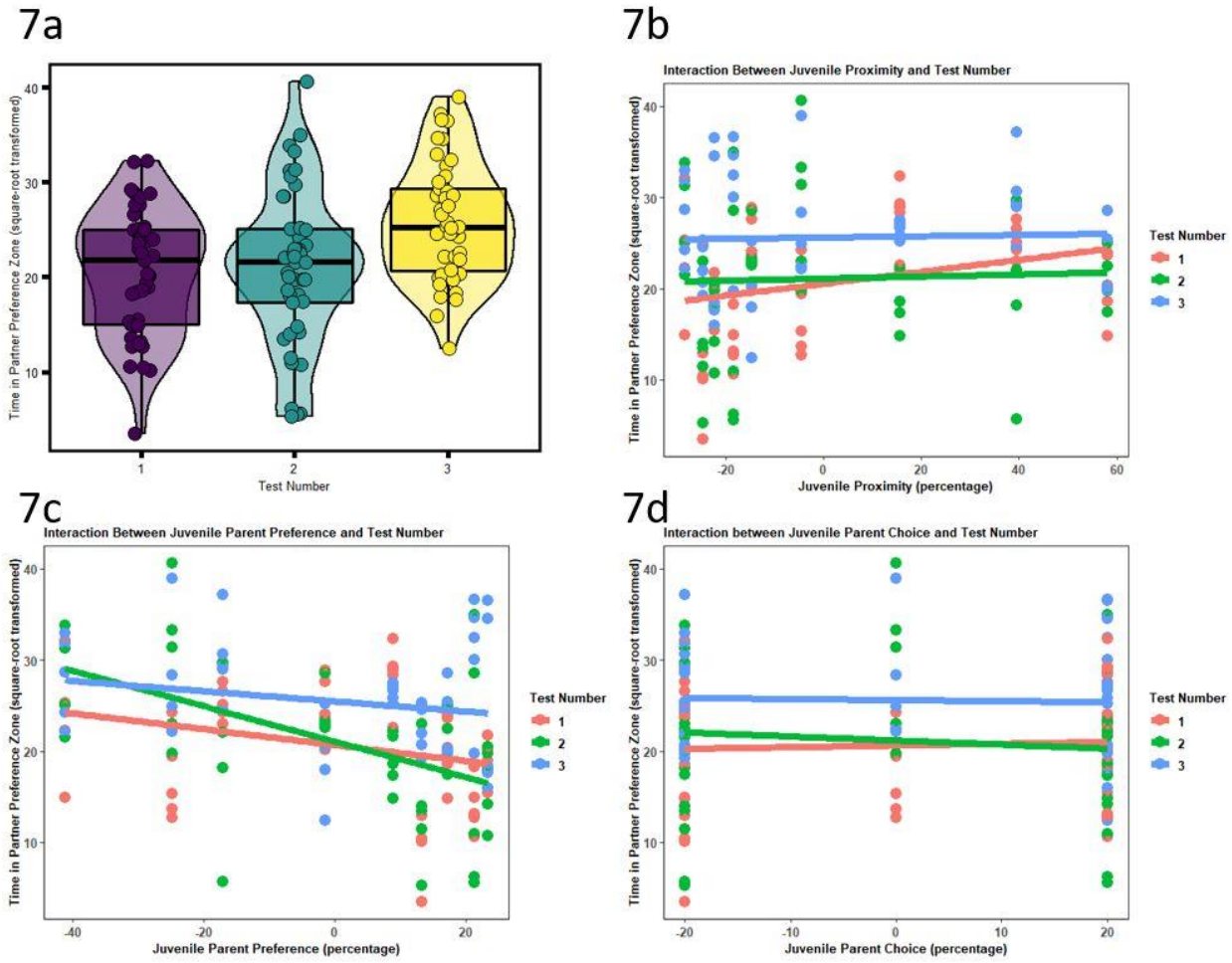


Figure 7. Partner Zone duration: a) the main effect of *Test Number*, interaction effects between *Test Number* and b) *Juvenile Proximity*, c) *Juvenile Parent Preference*, and d) *Juvenile Parent Choice.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

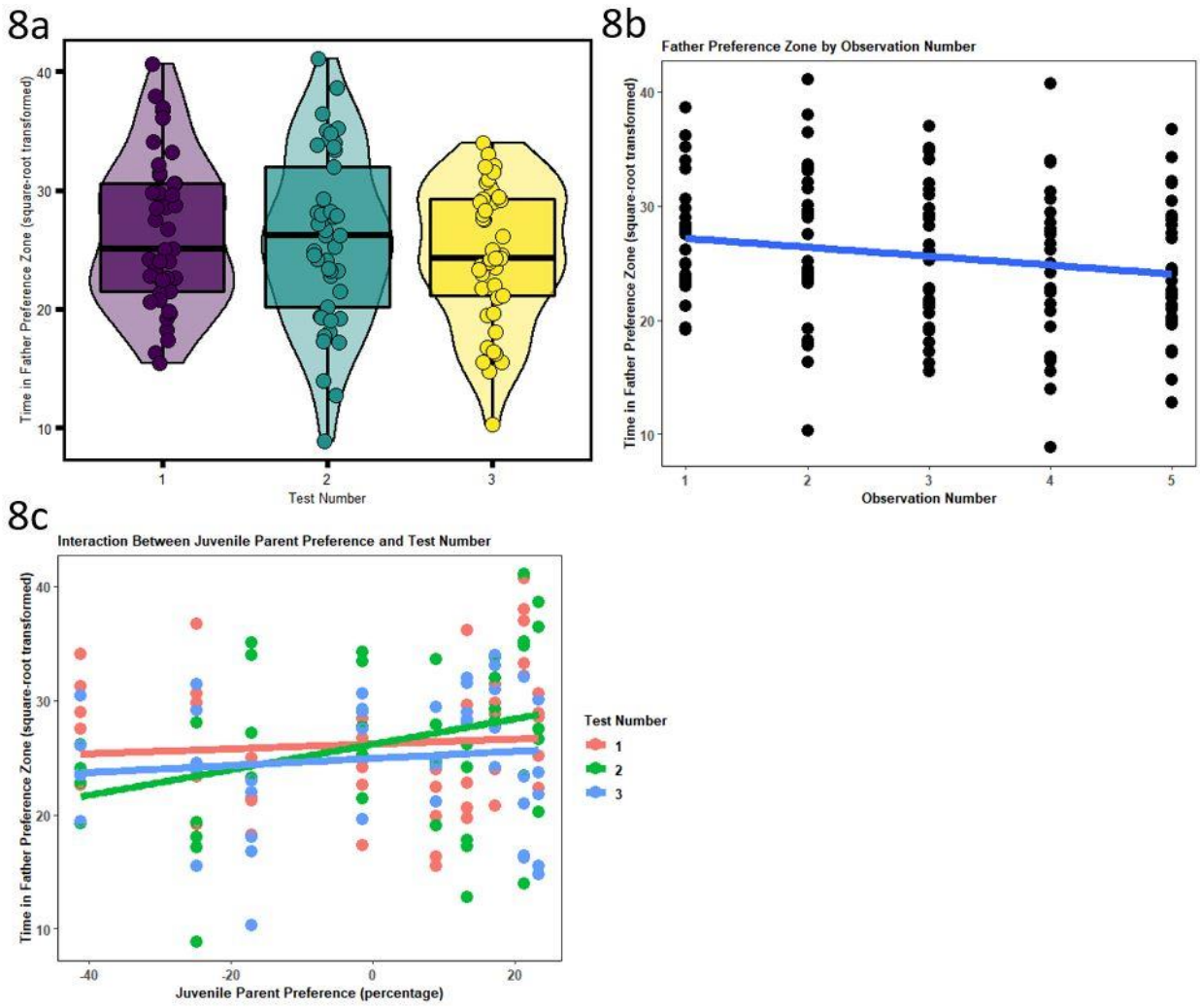


Figure 8. Father Zone duration: a) the main effect of *Test Number*, b) *Observation Number*, and interaction effect between *Test Number* and c) *Juvenile Parent Preference.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

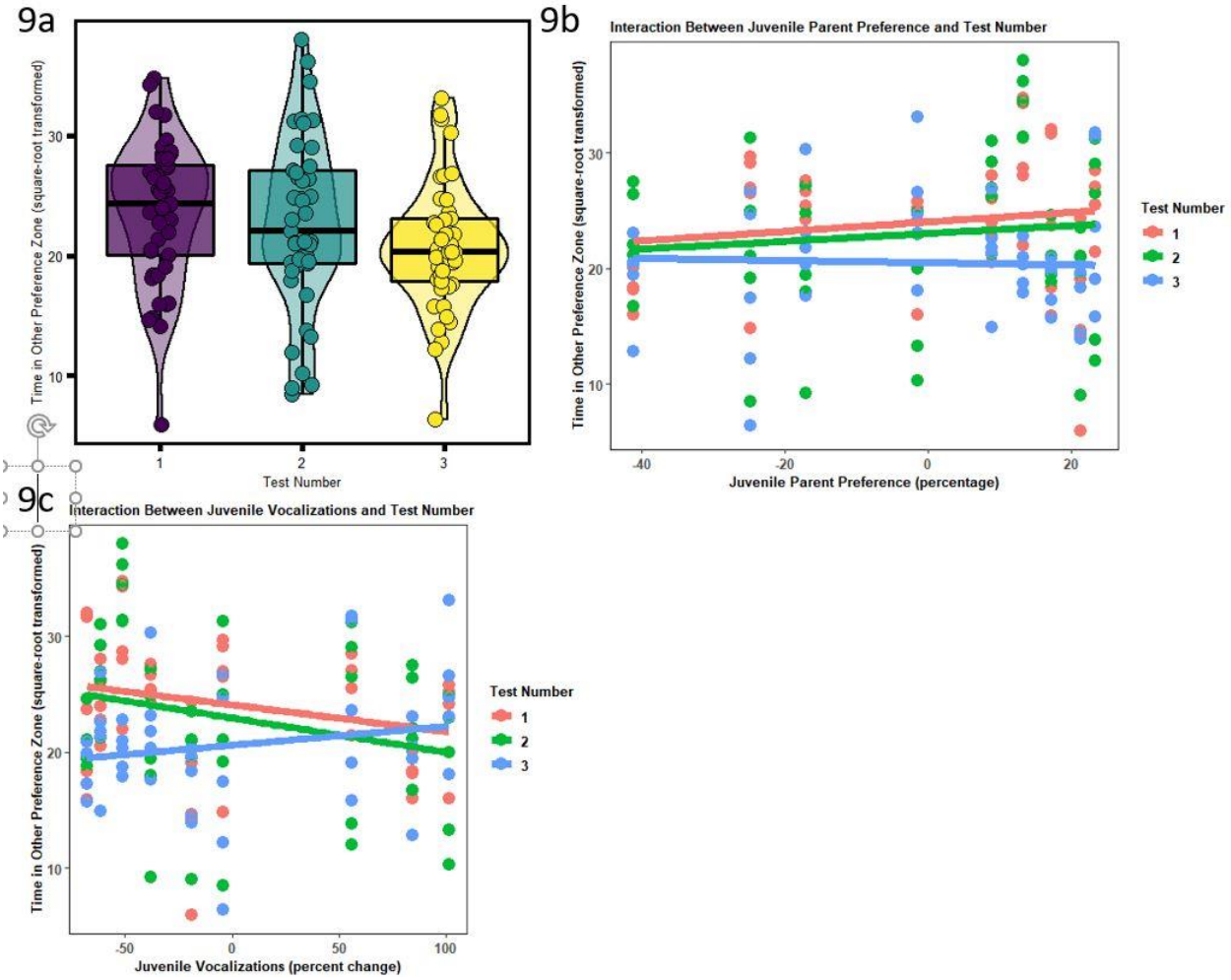


Figure 9. Other Zone duration: a) the main effect of *Test Number*, interaction effect between *Test Number* and b) *Juvenile Parent Preference*, and c) *Juvenile Vocalizations.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

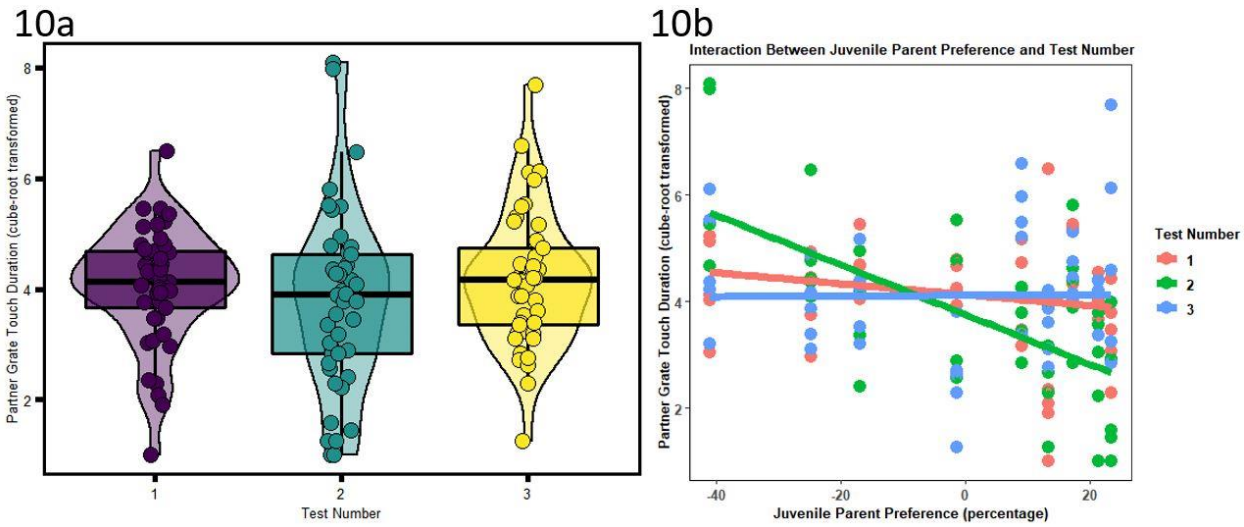


Figure 10. Partner Grate Touch duration: a) the main effect of *Test Number*, and interaction effect between *Test Number* and b) *Juvenile Parent Preference.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

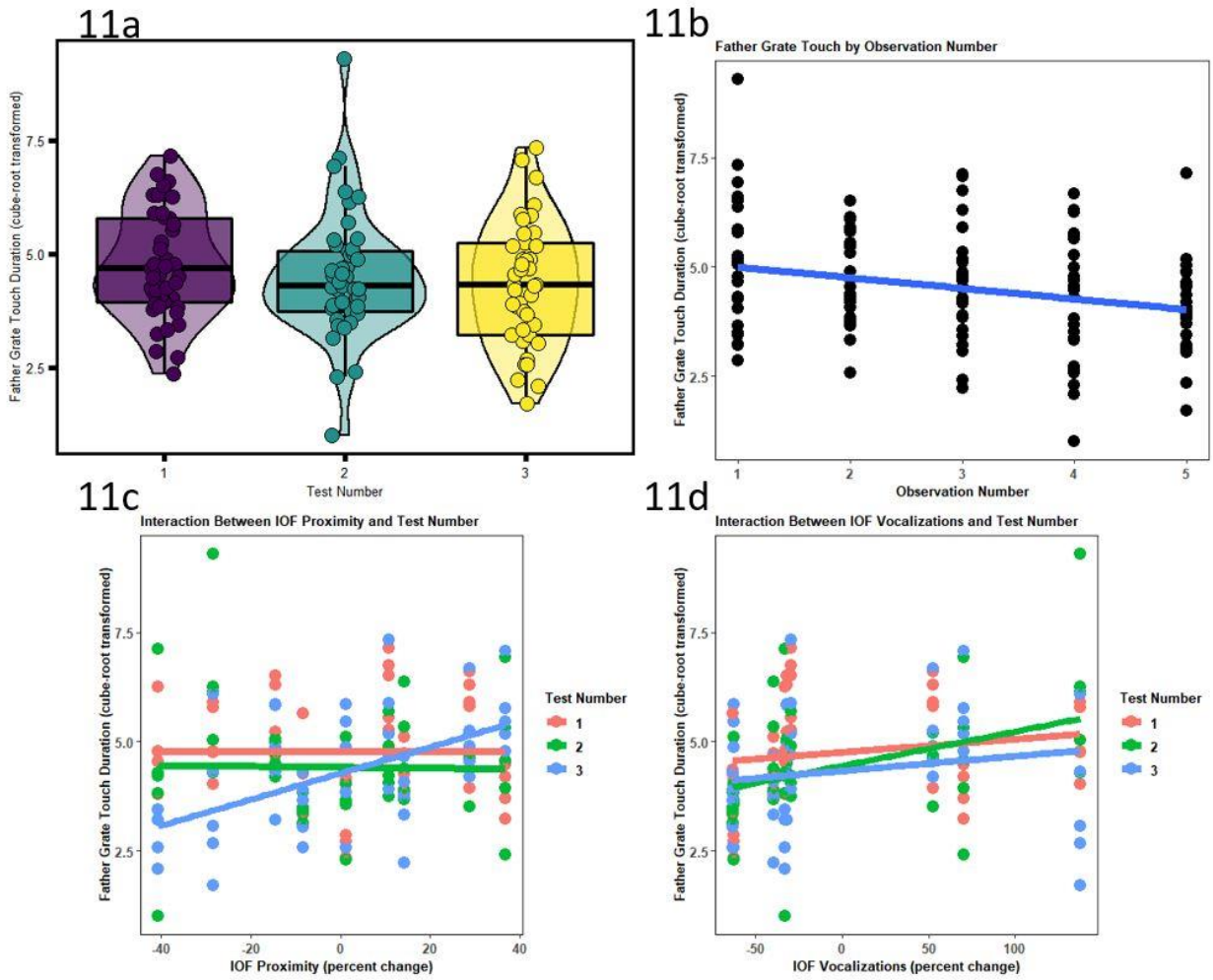


Figure 11. Father Grate Touch duration: a) the main effect of *Test Number*, b) *Observation Number*, interaction effects between *Test Number* and c) *IOF Proximity*, and d) *IOF Vocalization.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

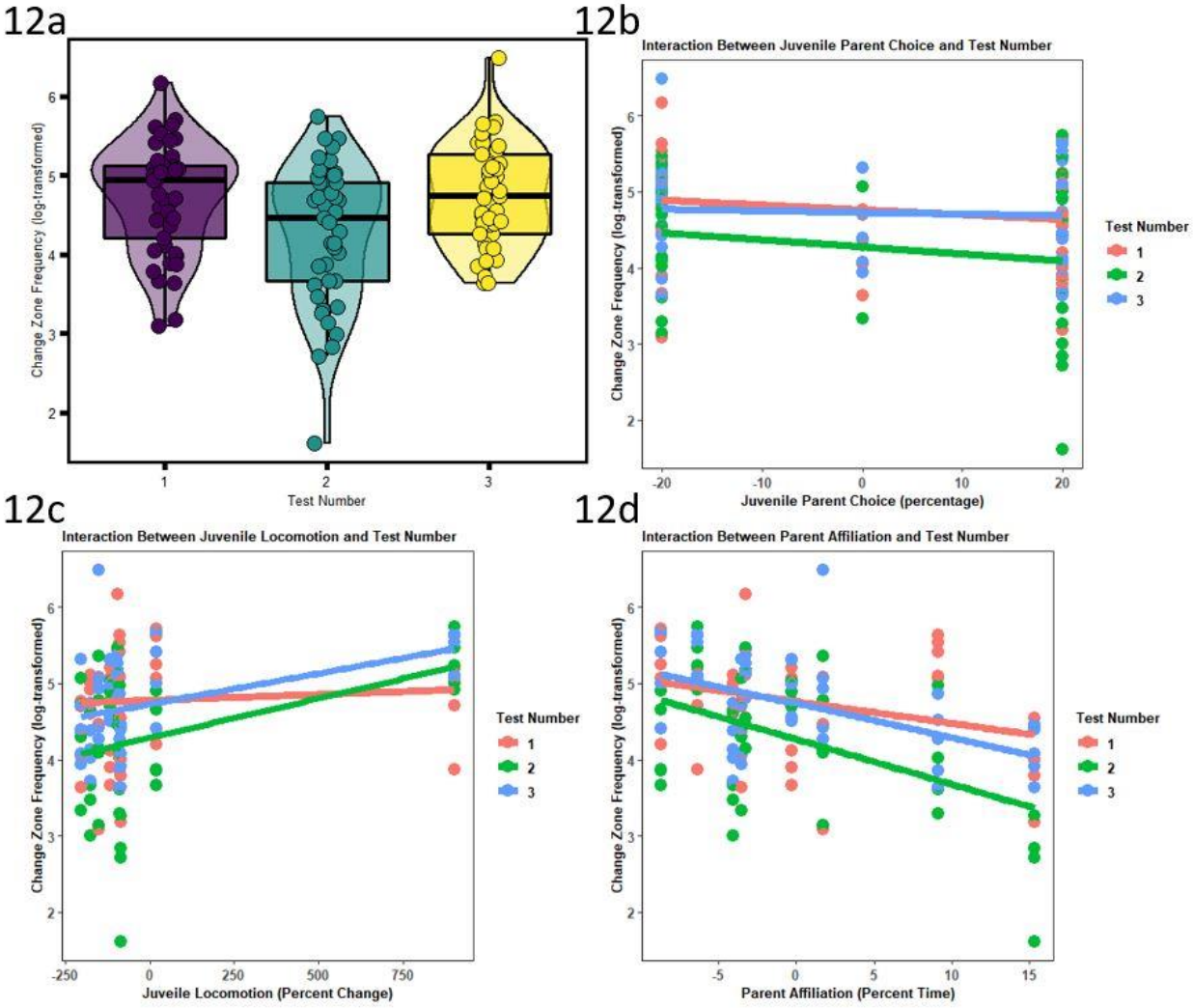


Figure 12. Zone Change frequency: a) the main effect of *Test Number*, interaction effect between *Test Number* and b) *Juvenile Parent Choice*, c) *Juvenile Locomotion*, and d) *Parent Affiliation.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

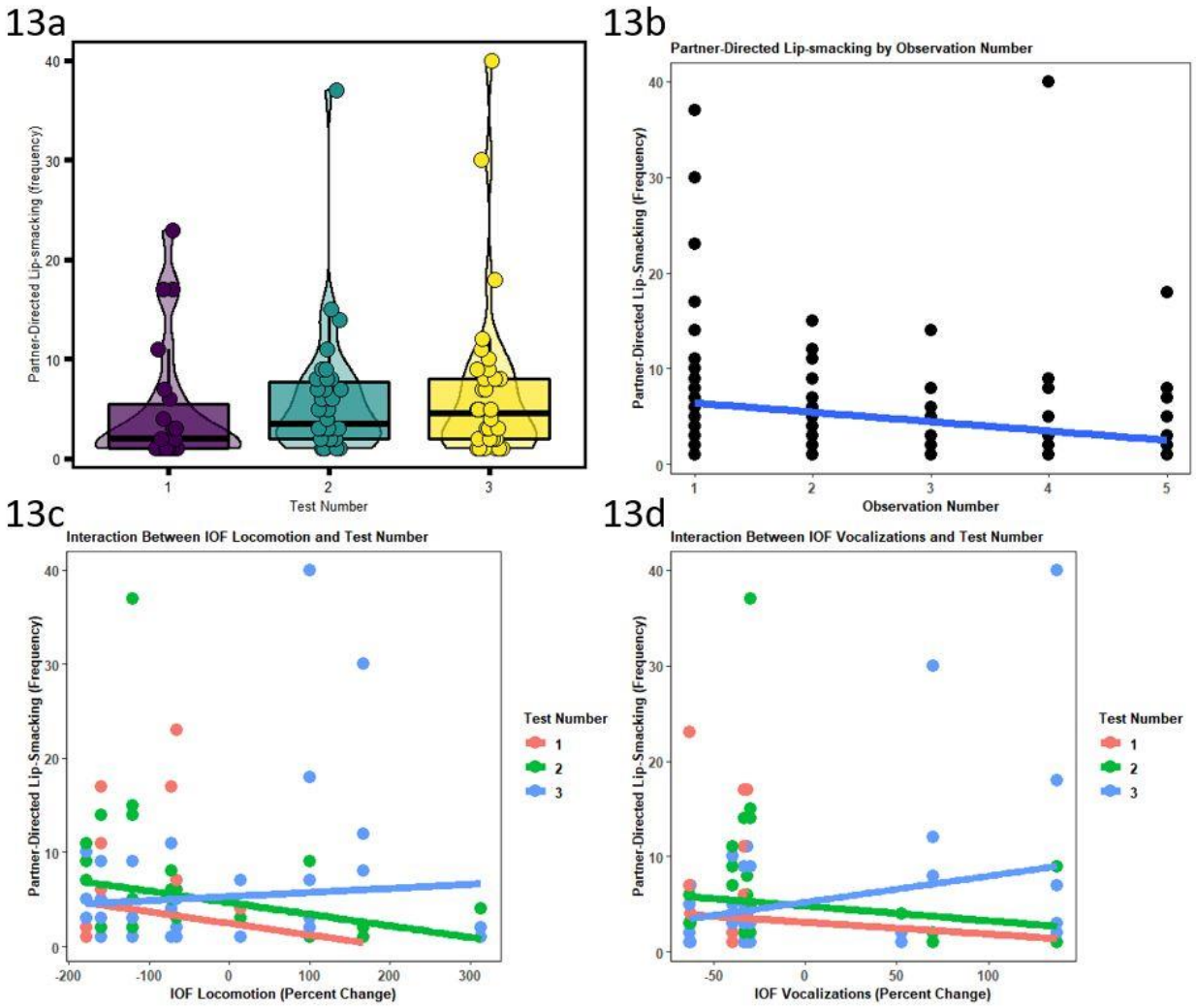


Figure 13. Partner-directed Lip-smacking frequency: a) the main effect of *Test Number*, b) *Observation Number*, interaction effects between *Test Number* and c) *IOF Locomotion*, and d) *IOF Vocalizations.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

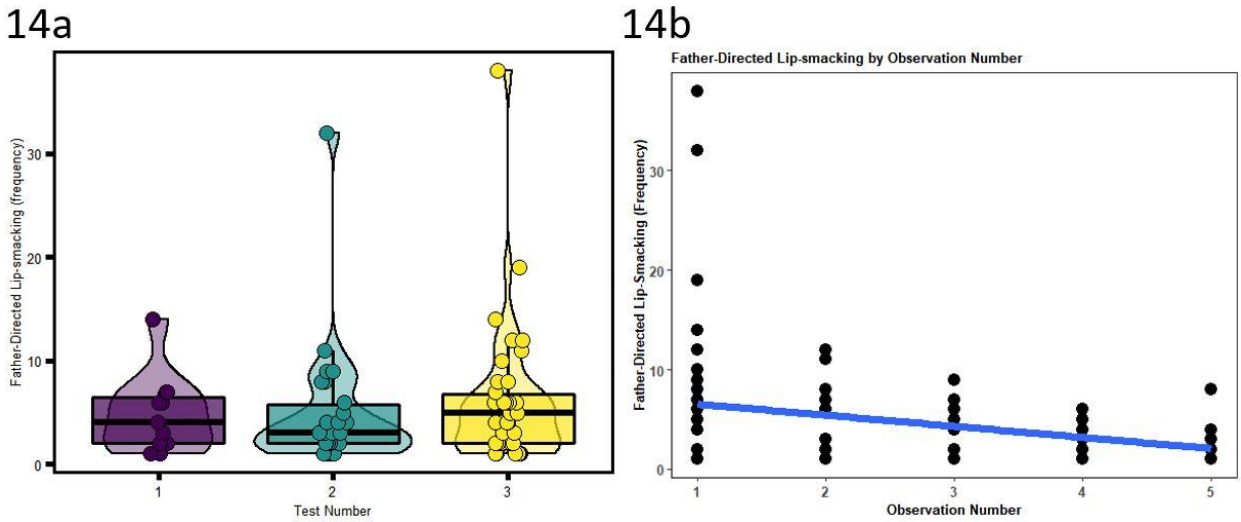


Figure 14. Father-directed Lip-smacking frequency: a) the main effect of *Test Number* and b) *Observation Number*. Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

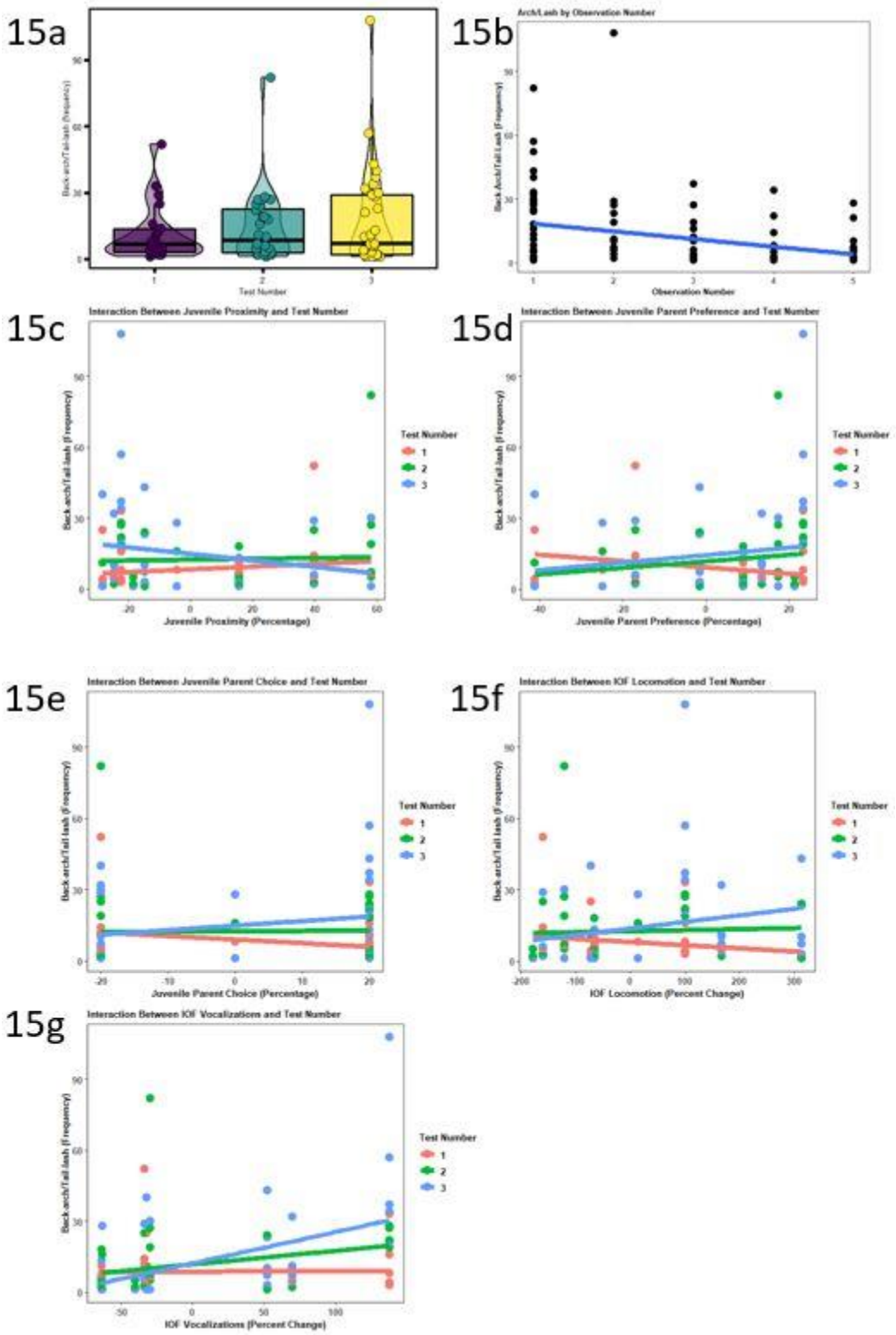


Figure 15. Back-arching/Tail-lashing frequency: a) the main effect of *Test Number*, b) *Observation Number*, interaction effects between *Test Number* and c) *Juvenile Proximity*, d)

Juvenile Parent Preference, e) *Juvenile Parent Choice*, f) *IOF Locomotion*, and g) *IOF Vocalizations*. Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

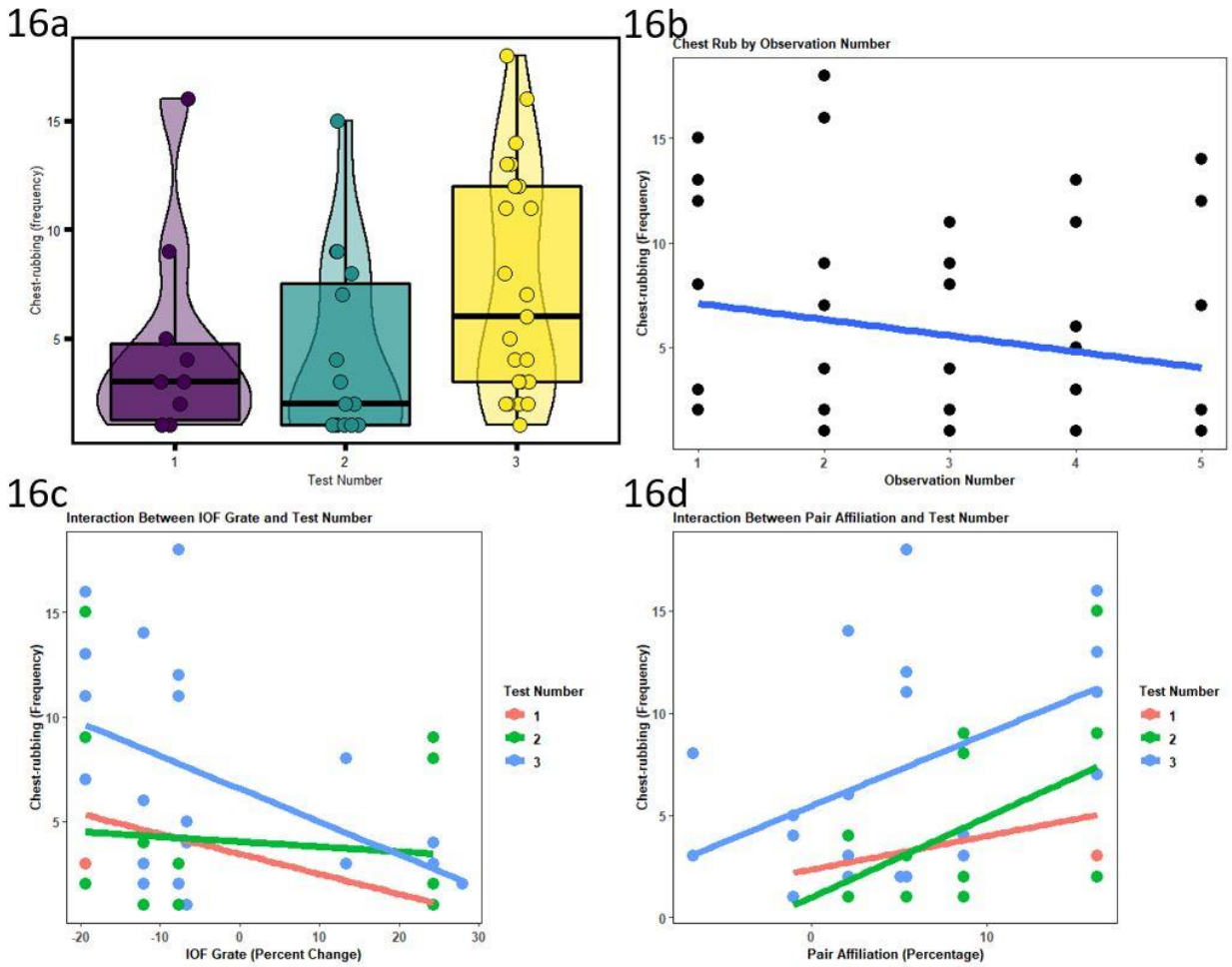


Figure 16. Chest Rubbing frequency: a) the main effect of *Test Number*, b) *Observaton Number*, interaction effects between *Test Number* and c) *IOF Grate* and d) *Pair Affiliation.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

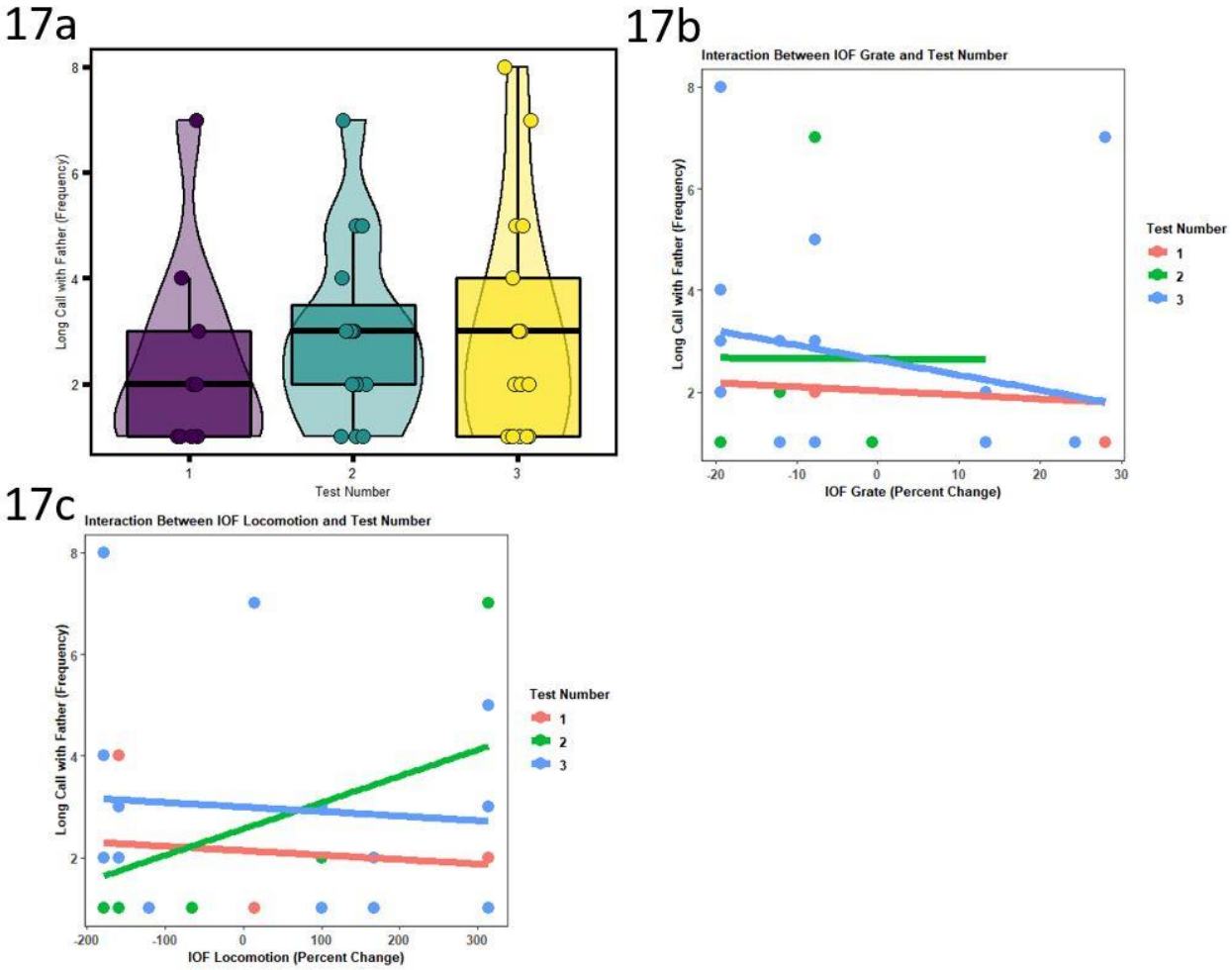


Figure 17. Long call with Father frequency: a) the main effect of *Test Number*, interaction effects between *Test Number* and b) *IOF Grate*, and c) *IOF Locomotion.* Test Number 1 = 1-week pre-pairing; Test Number 2 = 1-week post-pairing; Test Number 3 = 6-months post-pairing.

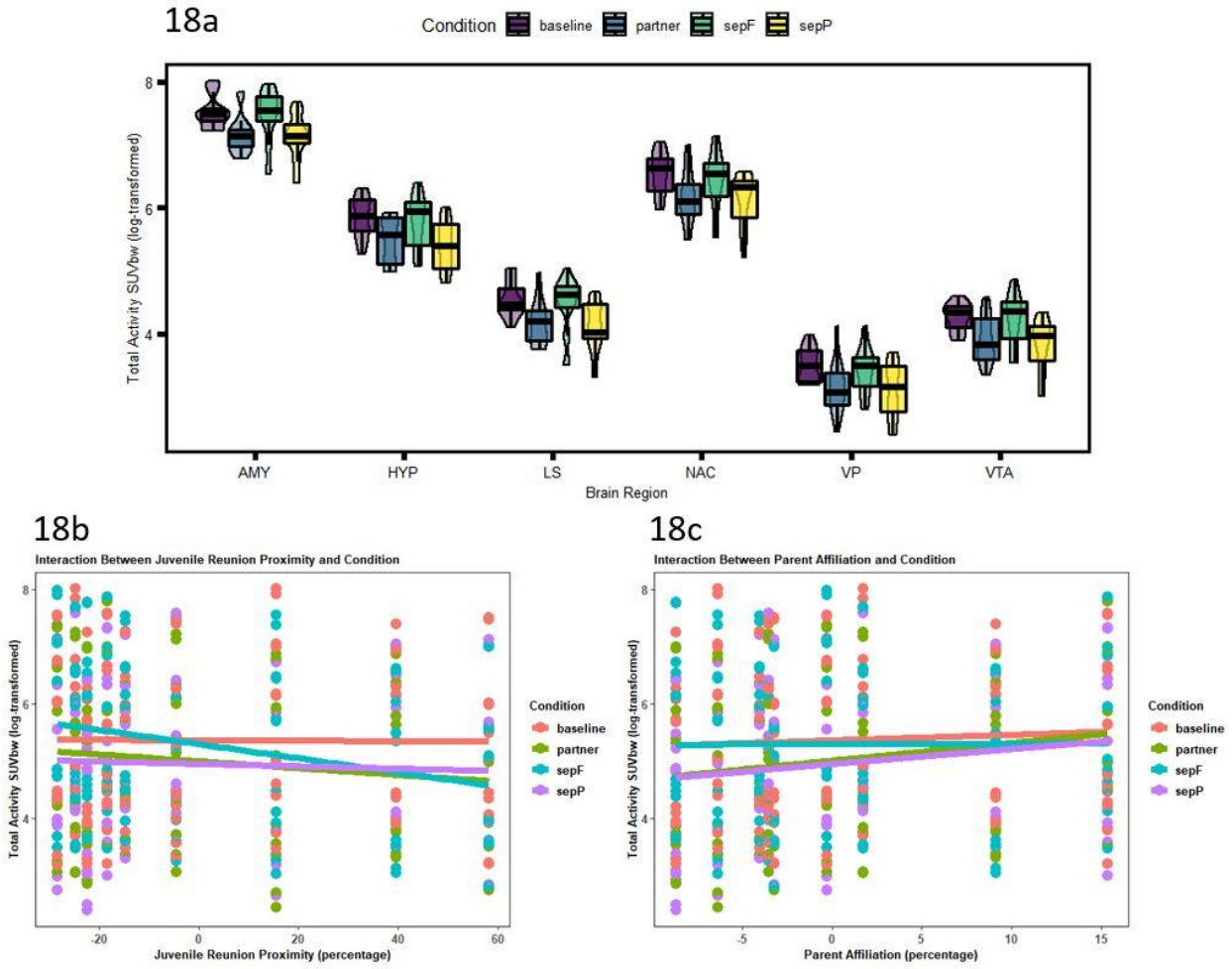


Figure 18. Social Salience Network glucose uptake (SUVbw): a) the main effect of *Condition*, interaction effects between *Condition* and b) *Juvenile Proximity*, and c) *Parent Affiliation.* baseline = when tested with father when still in natal group; partner = when tested with partner 6-months post-pairing; sepF = when separated from father while still in natal group; sepP = when separated from partner 6-months post-pairing; AMY = Amygdala; HYP = Hypothalamus; LS = lateral septum; NAC = nucleus accumbens; VP = ventral pallidum; VTA = ventral tegmental area.

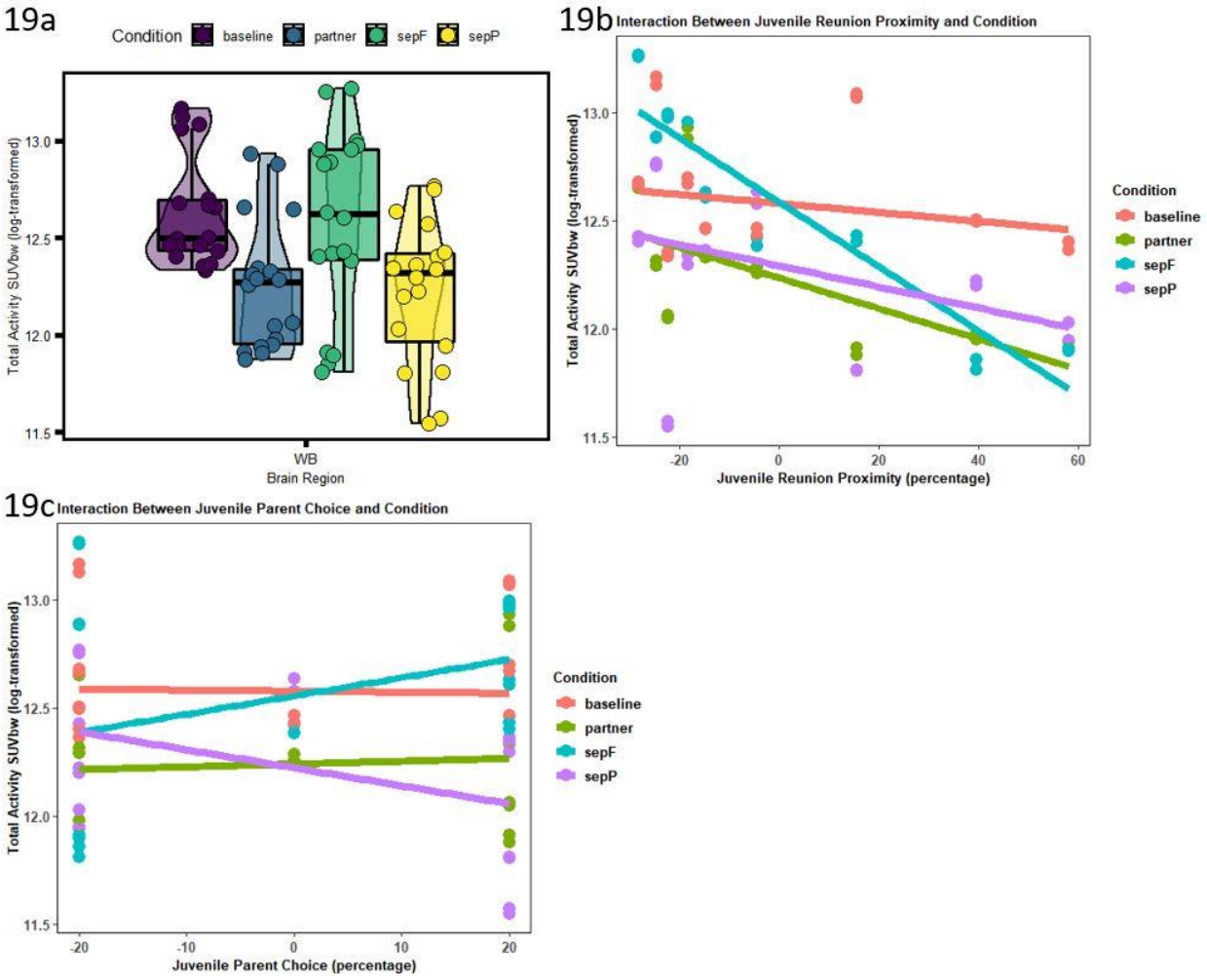


Figure 19. Whole Brain glucose uptake (SUVbw): a) the main effect of *Condition*, interaction effects between *Condition* and b) *Juvenile Proximity*, and c) *Parent Affiliation.* baseline = when tested with father when still in natal group; partner = when tested with partner 6-months post-pairing; sepF = when separated from father while still in natal group; sepP = when separated from partner 6-months post-pairing; WB = Whole Brain.

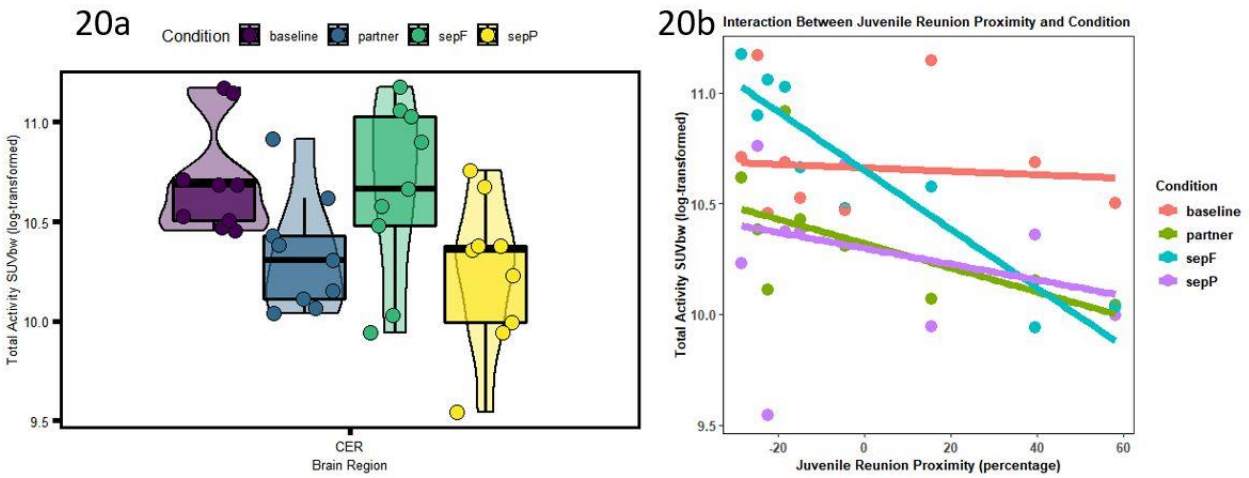


Figure 20. Cerebellum glucose uptake (SUVbw): a) the main effect of *Condition* and interaction effect between *Condition* and b) *Juvenile Proximity.* baseline = when tested with father when still in natal group; partner = when tested with partner 6-months post-pairing; sepF = when separated from father while still in natal group; sepP = when separated from partner 6-months post-pairing; CER = Cerebellum.

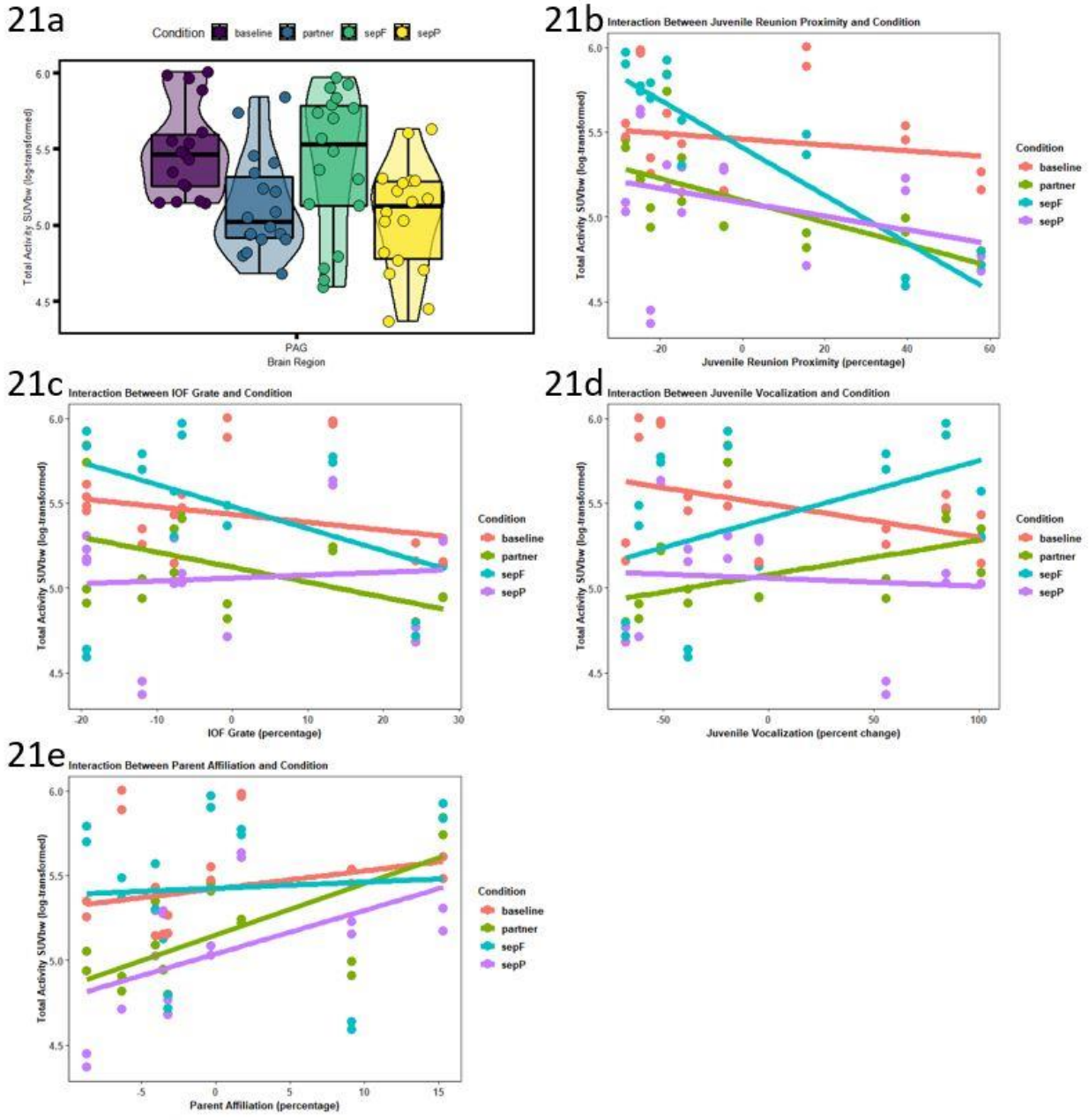


Figure 21. Periaqueductal Gray (PAG) glucose uptake (SUVbw): a) the main effect of *Condition*, interaction effects between *Condition* and b) *Juvenile Proximity*, c) *IOF Grate*, d) *Juvenile Vocalizations*, and e) *Parent Affiliation.* baseline = when tested with father when still in natal group; partner = when tested with partner 6-months post-pairing; sepF = when separated from father while still in natal group; sepP = when separated from partner 6-months post-pairing; PAG = Periaqueductal Gray.

SUPPLEMENTARY TABLES & FIGURES

Supplementary Table 1. Experiment 1 Log likelihood ratio test results comparing model fit for general linear mixed-effects models and zero-inflated mixed-effects models.

Supplementary 1a. Zone Ratio								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ZoneRatioonlyTestNo	5	2110.5	2125.1	-1050.3	2100.5			
ZoneRatioonlysig	6	2103.8	2121.2	-1045.9	2091.8	8.7101	1	0.003164
ZoneRationoInt	8	2104.4	2127.6	-1044.2	2088.4	3.435	2	0.179517
ZoneRationoPartEXP	14	2111.4	2152	-1041.7	2083.4	5.0173	6	0.541598
ZoneRationoObs	15	2113.3	2156.9	-1041.7	2083.3	0.0266	1	0.870528
ZoneRatioAll	16	2112.8	2159.3	-1040.4	2080.8	2.5363	1	0.111257
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	2114.9	2123.6	-1054.5	2108.9			
ZoneRatioonlysig	6	2103.8	2121.2	-1045.9	2091.8	17.093	3	0.0006764

Supplementary 1b. Percent Social								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PropSocialonlyTestNo	5	1112.8	1127.3	-551.39	1102.8			
PropSocialonlysig	7	1116	1136.3	-551.01	1102	0.7682	2	0.6810726
PropSocialnoInt	12	1104.1	1139	-540.04	1080.1	21.9256	5	0.0005409
PropSocialnoPartEXP	26	1106.4	1182	-527.22	1054.4	25.6457	14	0.0287029
PropSocialnoObs	27	1108.3	1186.8	-527.17	1054.3	0.1074	1	0.7430718
PropSocialAll	28	1106.3	1187.7	-525.17	1050.3	3.9937	1	0.0456715
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	1116.6	1125.3	-555.29	1110.6			
PropSocialnoPartEXP	26	1106.4	1182	-527.22	1054.4	56.146	23	0.0001351

Supplementary 1c. Partner Zone								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PPZonlyTestNo	5	907.95	922.47	-448.97	897.95			
PPZnoInt	8	895.81	919.05	-439.9	879.81	18.1428	3	0.000411
PPZnoPartEXP	14	897.22	937.89	-434.61	869.22	10.5902	6	0.101898
PPZnoObs	15	898.87	942.45	-434.43	868.87	0.3492	1	0.554564
PPZAll	16	900.28	946.77	-434.14	868.28	0.5813	1	0.445798
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	920.46	929.18	-457.23	914.46			
PPZnoInt	8	895.81	919.05	-439.9	879.81	34.657	5	1.76E-06

Supplementary 1d. Father Zone								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
FPZonlysig	5	884.36	898.89	-437.18	874.36			
FPZonlyTestNo	5	890.35	904.88	-440.18	880.35	0	0	
FPZnoInt	8	885.56	908.81	-434.78	869.56	10.7903	3	0.01292
FPZnoPartEXP	12	886.64	921.51	-431.32	862.64	6.9211	4	0.14011
FPZAll	13	887.97	925.74	-430.98	861.97	0.6749	1	0.41136
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	888.18	896.9	-441.09	882.18			
FPZonlysig	5	884.36	898.89	-437.18	874.36	7.8152	2	0.02009

Supplementary 1e. Other Zone

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ODonlyTestNo	5	870.15	884.67	-430.07	860.15			
ODonlysig	7	873.5	893.84	-429.75	859.5	0.6437	2	0.7247889
ODnoInt	12	862.69	897.55	-419.35	838.69	20.8114	5	0.0008793
ODnoPartEXPorObsNo	26	870.34	945.87	-409.17	818.34	20.3536	14	0.1193811
ODAll	28	872.14	953.49	-408.07	816.14	2.1919	2	0.3342272
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	872.55	881.27	-433.28	866.55			
ODnoInt	12	862.69	897.55	-419.35	838.69	27.865	9	0.001005

Supplementary 1f. Partner Grate Touch

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PGTDonlyTestNo	5	451.49	466.01	-220.74	441.49			
PGTDonlyPropPar	8	430.72	453.97	-207.36	414.72	26.7639	3	6.60E-06
PGTDoneInt	12	426.22	461.09	-201.11	402.22	12.5014	4	0.01399
PGTDnoPartEXP	20	435.35	493.45	-197.67	395.35	6.8744	8	0.55024
PGTDnoObs	21	437.24	498.25	-197.62	395.24	0.1088	1	0.7415
PGTDAll	22	438.7	502.62	-197.35	394.7	0.5395	1	0.46265
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	450.17	458.89	-222.09	444.17			
PGTDoneInt	12	426.22	461.09	-201.11	402.22	41.948	9	3.36E-06

Supplementary 1g. Father Grate Touch

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
FGTDonlyObsNo	4	438.24	449.86	-215.12	430.24			
FGTDonlyTestNo	5	450.61	465.14	-220.31	440.61	0	1	1
FGTDnoRel	6	438.83	456.27	-213.42	426.83	13.778	1	0.0002057
FGTDonlySig	12	426.43	461.29	-201.22	402.43	24.4056	6	0.0004397
FGTDsigInt	18	424.86	477.16	-194.43	388.86	13.5683	6	0.0348487
FGTDnoPartEXP	24	427.75	497.47	-189.87	379.75	9.1126	6	0.1673444
FGTDAll	25	429.7	502.33	-189.85	379.7	0.0495	1	0.8238596
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	449.67	458.38	-221.84	443.67			
FGTDsigInt	18	424.86	477.16	-194.43	388.86	54.808	15	1.92E-06

Supplementary 1h. Zone Change

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
logChangeZoneonlyTestNo	5	280.04	294.57	-135.02	270.04			
logChangeZonenoInt	8	264.06	287.3	-124.03	248.06	21.9848	3	6.57E-05
logChangeZonenoPartEXP	14	264.36	305.03	-118.18	236.36	11.699	6	0.06903
logChangeZonenoObs	15	266.19	309.77	-118.09	236.19	0.1703	1	0.67988
logChangeZoneAll	16	265.79	312.27	-116.89	233.79	2.3998	1	0.12135
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	293.69	302.41	-143.85	287.69			
logChangeZonenoInt	8	264.06	287.3	-124.03	248.06	39.635	5	1.77E-07

Supplementary 1i. Partner-directed Lip-smacking						
	AIC	BIC	log.Lik	LRT	df	p.value
Model1	762.69	767.62	-356.35			
Model2	763.76	768.3	-358.88	5.07	2	0.0794
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	763.76	768.3	-358.88			
Model3	765.63	769.78	-361.82	5.88	2	0.053
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	765.63	769.78	-361.82			
Model4	766.71	769.08	-371.36	19.08	9	0.0245
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	765.63	769.78	-361.82			
Model5	765	768.36	-365.5	7.37	4	0.1176
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	765	768.36	-365.5			
Model6	763.79	766.75	-366.89	2.79	2	0.2484
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	763.79	766.75	-366.89			
Model7	762.7	765.47	-367.35	0.92	1	0.3386
	AIC	BIC	log.Lik	LRT	df	p.value
Model7	762.7	765.47	-367.35			
Model8	766.71	769.08	-371.36	8.01	2	0.0182
	AIC	BIC	log.Lik	LRT	df	p.value
Model7	762.7	765.47	-367.35			
Model9	762.56	764.92	-369.28	3.85	2	0.1457
	AIC	BIC	log.Lik	LRT	df	p.value
Model9	762.56	764.92	-369.28			
Model10	879.62	881.59	-429.81	121.06	2	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model9	762.56	764.92	-369.28			
Model11	961.8	962.39	-477.9	217.24	9	<0.0001

Supplementary 1j. Father-directed Lip-smacking

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	446.65	451.58	-198.32			
Model2	448.85	453.39	-201.43	6.21	2	0.0449
	AIC	BIC	log.Lik	LRT	df	p.value
Model1	446.65	451.58	-198.32			
Model3	445.49	449.43	-202.74	8.84	5	0.1156
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	445.49	449.43	-202.74			
Model4	454.89	458.45	-209.45	13.41	2	0.0012
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	445.49	449.43	-202.74			
Model5	444.98	448.73	-203.49	1.49	1	0.222
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model6	763.79	766.75	-366.89	326.81	4	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model7	762.7	765.47	-367.35	327.72	5	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model8	766.71	769.08	-371.36	335.73	7	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model9	454.94	458.29	-210.47	13.96	2	9.00E-04
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model10	511.25	514.4	-239.62	72.27	3	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	444.98	448.73	-203.49			
Model11	961.8	962.39	-477.9	548.82	16	<0.0001

Supplementary 1k. Back-arching/Tail-lashing

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	869.08	877.56	-391.54			
Model2	862.96	871.04	-390.48	2.12	2	0.346
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	862.96	871.04	-390.48			
Model3	856.02	862.14	-397.01	13.07	10	0.2198
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	856.02	862.14	-397.01			
Model4	855.58	861.5	-397.79	1.56	1	0.2121
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	855.58	861.5	-397.79			
Model5	853.67	859.39	-397.84	0.09	1	0.7646
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	853.67	859.39	-397.84			
Model6	855.12	860.44	-400.56	5.45	2	0.0656
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	855.12	860.44	-400.56			
Model7	886.7	891.04	-421.35	41.58	5	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	855.12	860.44	-400.56			
Model8	851.13	856.26	-399.57	1.99	1	0.1588
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	851.13	856.26	-399.57			
Model9	1531.89	1533.47	-757.95	716.76	18	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	851.13	856.26	-399.57			
Model10	1560.62	1561.21	-777.31	755.49	23	<0.0001

Supplementary 1l. Chest Rubbing

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	402.35	407.28	-176.17			
Model2	399.75	404.29	-176.88	1.41	2	0.4946
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	399.75	404.29	-176.88			
Model3	395.93	400.07	-176.96	0.17	2	0.917
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	395.93	400.07	-176.96			
Model4	392.77	396.52	-177.39	0.85	2	0.6554
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	392.77	396.52	-177.39			
Model5	395.72	399.07	-180.86	6.94	2	0.031
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	392.77	396.52	-177.39			
Model6	418.7	422.25	-191.35	27.93	1	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	392.77	396.52	-177.39			
Model7	424.4	426.96	-199.2	43.63	6	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	392.77	396.52	-177.39			
Model8	391.27	394.82	-177.63	0.5	1	0.4814
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	391.27	394.82	-177.63			
Model9	402.31	405.07	-187.16	19.05	4	8.00E-04
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	391.27	394.82	-177.63			
Model10	429.88	431.66	-205.94	56.62	9	<0.0001
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	391.27	394.82	-177.63			
Model11	470.56	471.15	-232.28	109.29	15	<0.0001

Supplementary 1m. Long call with Father

	AIC	BIC	log.Lik	LRT	df	p.value
Model1	323.44	328.37	-136.72			
Model2	319.11	323.64	-136.55	0.33	2	0.8481
	AIC	BIC	log.Lik	LRT	df	p.value
Model2	319.11	323.64	-136.55			
Model3	320.08	324.42	-138.04	2.97	1	0.0847
	AIC	BIC	log.Lik	LRT	df	p.value
Model3	320.08	324.42	-138.04			
Model4	313.93	317.87	-136.96	2.15	2	0.3413
	AIC	BIC	log.Lik	LRT	df	p.value
Model4	313.93	317.87	-136.96			
Model5	313.66	317.21	-138.83	3.73	2	0.155
	AIC	BIC	log.Lik	LRT	df	p.value
Model5	313.66	317.21	-138.83			
Model6	312.66	315.62	-141.33	5	3	0.1719
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	312.66	315.62	-141.33			
Model7	321.28	323.84	-147.64	12.62	2	0.0018
	AIC	BIC	log.Lik	LRT	df	p.value
Model6	312.66	315.62	-141.33			
Model8	311.02	313.78	-141.51	0.36	1	0.5458
	AIC	BIC	log.Lik	LRT	df	p.value
Model8	311.02	313.78	-141.51			
Model9	308.73	311.3	-141.37	0.29	1	0.5924
	AIC	BIC	log.Lik	LRT	df	p.value
Model9	308.73	311.3	-141.37			
Model10	311.4	313.57	-144.7	6.67	2	0.0357
	AIC	BIC	log.Lik	LRT	df	p.value
Model9	308.73	311.3	-141.37			
Model11	312.96	313.94	-151.48	20.22	8	0.0095
	AIC	BIC	log.Lik	LRT	df	p.value
Model9	308.73	311.3	-141.37			
Model12	319.47	320.07	-156.74	30.74	10	6.00E-04

Supplementary 1a Models:

ZoneRatioonlyTestNo: ZoneRatio ~ TestNo + (1 | ID)

ZoneRatioonlysig: ZoneRatio ~ TestNo + PropPar + (1 | ID)

ZoneRationoInt: ZoneRatio ~ TestNo + PropPar + CnRPar + JuvVoc + (1 | ID)

ZoneRationoPartEXP: ZoneRatio ~ TestNo + PropPar + CnRPar + JuvVoc + PropPar * TestNo +
CnRPar * TestNo + JuvVoc * TestNo + (1 | ID)

ZoneRationoObs: ZoneRatio ~ TestNo + PartnerEXP + PropPar + CnRPar + JuvVoc +
PropPar * TestNo + CnRPar * TestNo + JuvVoc * TestNo + (1 |
ID)

ZoneRatioAll: ZoneRatio ~ TestNo + ObsNo + PartnerEXP + PropPar + CnRPar +
JuvVoc + PropPar * TestNo + CnRPar * TestNo + JuvVoc * TestNo +
(1 | ID)

Null1: ZoneRatio ~ 1 + (1 | ID)

Supplementary 1b Models:

PropSocialonlyTestNo: PropSocial ~ TestNo + (1 | ID)

PropSocialonlysig: PropSocial ~ TestNo + PropPar + JuvVoc + (1 | ID)

PropSocialnoInt: PropSocial ~ TestNo + PropPar + CnRPar + InfCarry + IOFGrate +
IOFLine + IOFVoc + JuvVoc + (1 | ID)

PropSocialnoPartEXP: PropSocial ~ TestNo + PropPar + CnRPar + InfCarry + IOFGrate +
IOFLine + IOFVoc + JuvVoc + PropPar * TestNo + CnRPar * TestNo +
InfCarry * TestNo + IOFGrate * TestNo + IOFLine * TestNo +
IOFVoc * TestNo + JuvVoc * TestNo + (1 | ID)

PropSocialnoObs: PropSocial ~ TestNo + PartnerEXP + PropPar + CnRPar + InfCarry +
IOFGrate + IOFLine + IOFVoc + JuvVoc + PropPar * TestNo +
CnRPar * TestNo + InfCarry * TestNo + IOFGrate * TestNo +
IOFLine * TestNo + IOFVoc * TestNo + JuvVoc * TestNo + (1 |
ID)

PropSocialAll: PropSocial ~ TestNo + ObsNo + PartnerEXP + PropPar + CnRPar +
InfCarry + IOFGrate + IOFLine + IOFVoc + JuvVoc + PropPar *
TestNo + CnRPar * TestNo + InfCarry * TestNo + IOFGrate *
TestNo + IOFLine * TestNo + IOFVoc * TestNo + JuvVoc * TestNo +
(1 | ID)

Null1: PropSocial ~ 1 + (1 | ID)

Supplementary 1c Models:

PPZonlyTestNo: sqrtPPZD ~ TestNo + (1 | ID)

PPZnoInt: sqrtPPZD ~ TestNo + JuvReun + PropPar + CnRPar + (1 | ID)

PPZnoPartEXP: sqrtPPZD ~ TestNo + JuvReun + PropPar + CnRPar + JuvReun * TestNo +
PropPar * TestNo + CnRPar * TestNo + (1 | ID)

PPZnoObs: sqrtPPZD ~ TestNo + PartnerEXP + JuvReun + PropPar + CnRPar +
JuvReun * TestNo + PropPar * TestNo + CnRPar * TestNo + (1 |
ID)

PPZAll: sqrtPPZD ~ TestNo + ObsNo + PartnerEXP + JuvReun + PropPar +
CnRPar + JuvReun * TestNo + PropPar * TestNo + CnRPar * TestNo +
(1 | ID)

Null1: sqrtPPZD ~ 1 + (1 | ID)

Supplementary 1d Models:

FPZonlysig: sqrtFPZD ~ ObsNo + PropPar + (1 | ID)

FPZonlyTestNo: sqrtFPZD ~ TestNo + (1 | ID)

FPZnoInt: sqrtFPZD ~ TestNo + ObsNo + PropPar + JuvLoc + (1 | ID)

FPZnoPartEXP: sqrtFPZD ~ TestNo + ObsNo + PropPar + JuvLoc + PropPar * TestNo +
JuvLoc * TestNo + (1 | ID)

FPZAll: sqrtFPZD ~ TestNo + ObsNo + PartnerEXP + PropPar + JuvLoc + PropPar *
TestNo + JuvLoc * TestNo + (1 | ID)

Null1: sqrtFPZD ~ 1 + (1 | ID)

Supplementary 1e Models:

ODonlyTestNo: sqrtOD ~ TestNo + (1 | ID)

ODonlysig: sqrtOD ~ TestNo + PropPar + JuvVoc + (1 | ID)

ODnoInt: sqrtOD ~ TestNo + PropPar + InfCarry + IOFGrate + IOFLine + IOFVoc +
JuvVoc + JuvLoc + (1 | ID)

ODnoPartEXPorObsNo: sqrtOD ~ TestNo + PropPar + InfCarry + IOFGrate + IOFLine + IOFVoc +
JuvVoc + JuvLoc + PropPar * TestNo + InfCarry * TestNo +
IOFGrate * TestNo + IOFLine * TestNo + IOFVoc * TestNo +
JuvVoc * TestNo + JuvLoc * TestNo + (1 | ID)

ODAll: sqrtOD ~ TestNo + ObsNo + PartnerEXP + PropPar + InfCarry + IOFGrate +
IOFLine + IOFVoc + JuvVoc + JuvLoc + PropPar * TestNo + InfCarry *
TestNo + IOFGrate * TestNo + IOFLine * TestNo + IOFVoc *
TestNo + JuvVoc * TestNo + JuvLoc * TestNo + (1 | ID)

Null1: sqrtOD ~ 1 + (1 | ID)

Supplementary 1f Models:

PGTDonlyTestNo: cubePGTD ~ TestNo + (1 | ID)

PGTDonlyPropPar: cubePGTD ~ TestNo + PropPar + PropPar * TestNo + (1 | ID)

PGTDoneInt: cubePGTD ~ TestNo + JuvReun + PropPar + IOFLine + JuvVoc + PairAff +
PropPar * TestNo + (1 | ID)

PGTDnoPartEXP: cubePGTD ~ TestNo + JuvReun + PropPar + IOFLine + JuvVoc + PairAff +
JuvReun * TestNo + PropPar * TestNo + IOFLine * TestNo +
JuvVoc * TestNo + PairAff * TestNo + (1 | ID)

PGTDnoObs: cubePGTD ~ TestNo + PartnerEXP + JuvReun + PropPar + IOFLine +
JuvVoc + PairAff + JuvReun * TestNo + PropPar * TestNo +
IOFLine * TestNo + JuvVoc * TestNo + PairAff * TestNo + (1 |

ID)

PGTDAll: cubePGTD ~ TestNo + ObsNo + PartnerEXP + JuvReun + PropPar +

IOFLine + JuvVoc + PairAff + JuvReun * TestNo + PropPar *

TestNo + IOFLine * TestNo + JuvVoc * TestNo + PairAff * TestNo +

(1 | ID)

Null1: cubePGTD ~ 1 + (1 | ID)

Supplementary 1g Models:

FGTDonlyObsNo: cubeFGTD ~ ObsNo + (1 | ID)

FGTDonlyTestNo: cubeFGTD ~ TestNo + (1 | ID)

FGTDnoRel: cubeFGTD ~ TestNo + ObsNo + (1 | ID)

FGTDonlySig: cubeFGTD ~ TestNo + ObsNo + IOFProx + IOFVoc + IOFProx * TestNo +

IOFVoc * TestNo + (1 | ID)

FGTDsigInt: cubeFGTD ~ TestNo + ObsNo + InfCarry + IOFProx + IOFVoc + JuvVoc +

JuvLoc + ParAff + IOFProx * TestNo + IOFVoc * TestNo + ParAff *

TestNo + (1 | ID)

FGTDnoPartEXP: cubeFGTD ~ TestNo + ObsNo + InfCarry + IOFProx + IOFVoc + JuvVoc +

JuvLoc + ParAff + InfCarry * TestNo + IOFProx * TestNo +

IOFVoc * TestNo + JuvVoc * TestNo + JuvLoc * TestNo + ParAff *

TestNo + (1 | ID)

FGTDAll: cubeFGTD ~ TestNo + ObsNo + PartnerEXP + InfCarry + IOFProx +

IOFVoc + JuvVoc + JuvLoc + ParAff + InfCarry * TestNo + IOFProx *

TestNo + IOFVoc * TestNo + JuvVoc * TestNo + JuvLoc * TestNo +

ParAff * TestNo + (1 | ID)

Null1: cubeFGTD ~ 1 + (1 | ID)

Supplementary 1h Models:

logChangeZoneonlyTestNo: logChangeZone ~ TestNo + (1 | ID)

logChangeZonenoInt: logChangeZone ~ TestNo + CnRPar + JuvLoc + ParAff + (1 | ID)

logChangeZonenoPartEXP: logChangeZone ~ TestNo + CnRPar + JuvLoc + ParAff + CnRPar *
TestNo + JuvLoc * TestNo + ParAff * TestNo + (1 | ID)

logChangeZonenoObs: logChangeZone ~ TestNo + PartnerEXP + CnRPar + JuvLoc + ParAff +
CnRPar * TestNo + JuvLoc * TestNo + ParAff * TestNo + (1 |
ID)

logChangeZoneAll: logChangeZone ~ TestNo + ObsNo + PartnerEXP + CnRPar + JuvLoc +
ParAff + CnRPar * TestNo + JuvLoc * TestNo + ParAff * TestNo +
(1 | ID)

Null1: logChangeZone ~ 1 + (1 | ID)

Supplementary 1i Models:

```
Model1<-mixed_model(PLS ~ TestNo + ObsNo + PartnerEXP +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo + ObsNo + PartnerEXP +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(PLS ~ TestNo + ObsNo + PartnerEXP +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
```

```
 family = zi.poisson(),
 zi_fixed = ~ TestNo + ObsNo + PartnerEXP +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 iter_EM = 0)
Model3<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 iter_EM = 0)
Model4<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
Model5<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
```

```
 family = zi.poisson(),
 zi_fixed = ~ TestNo +
 IOFVoc +
 IOFVoc*TestNo,
 iter_EM = 0)
Model6<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo +
 IOFVoc,
 iter_EM = 0)
Model7<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo,
 iter_EM = 0)
Model8<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
```

```
 zi_fixed = ~ 1,
 iter_EM = 0)
Model9<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc +
 IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo,
 iter_EM = 0)
Model10<-mixed_model(PLS ~ TestNo + ObsNo +
 IOFLine + IOFVoc,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ TestNo,
 iter_EM = 0)
Model11<-mixed_model(PLS ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1j Models:

```
Model1<-mixed_model(FLS ~ TestNo + ObsNo + PartnerEXP +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
```

```
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo + ObsNo + PartnerEXP +
 PropPar + IOFProx + PairAff,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(FLS ~ TestNo + ObsNo + PartnerEXP +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo + ObsNo + PartnerEXP +
 PropPar + IOFProx + PairAff,
 iter_EM = 0)
Model3<-mixed_model(FLS ~ TestNo + ObsNo + PartnerEXP +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model4<-mixed_model(FLS ~ TestNo + ObsNo + PartnerEXP +
```

```
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
Model5<-mixed_model(FLS ~ TestNo + ObsNo +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model6<-mixed_model(FLS ~ TestNo + ObsNo +
 PropPar + IOFProx + PairAff,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model7<-mixed_model(FLS ~ ObsNo,
```

```
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model8<-mixed_model(FLS ~ TestNo + ObsNo +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model9<-mixed_model(FLS ~ TestNo + ObsNo +
 PropPar + IOFProx + PairAff +
 PropPar*TestNo + IOFProx*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ 1,
 zi_random = ~1|ID,
 iter_EM = 0)
Model10<-mixed_model(FLS ~ TestNo + ObsNo +
 PropPar + PairAff +
```

```
 PropPar*TestNo +
 PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = FLS ~ TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model11<-mixed_model(FLS ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1k Models:

```
Model1<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
```

```
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 iter_EM = 0)
Model3<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc,
 iter_EM = 0)
Model4<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
```

```
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFVoc,
 iter_EM = 0)
Model5<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + IOFVoc,
 iter_EM = 0)
Model6<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ ObsNo + PartnerEXP +
 JuvReun + PropPar + IOFVoc,
 iter_EM = 0)
Model7<-mixed_model(Arch ~ TestNo + ObsNo + PartnerEXP +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
```

```
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ 1,
 iter_EM = 0)
Model8<-mixed_model(Arch ~ TestNo + ObsNo +
 JuvReun + PropPar + CnRPar + IOFLine + IOFVoc +
 JuvReun*TestNo + PropPar*TestNo + CnRPar*TestNo +
 IOFLine*TestNo + IOFVoc*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ ObsNo + PartnerEXP +
 JuvReun + PropPar + IOFVoc,
 iter_EM = 0)
Model9<-mixed_model(Arch ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Arch ~ ObsNo + PartnerEXP +
 JuvReun + PropPar + IOFVoc,
 iter_EM = 0)
Model10<-mixed_model(Arch ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
```

```
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1l Models:

```
Model1<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 iter_EM = 0)
Model3<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
```

```
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 iter_EM = 0)
Model4<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo,
 iter_EM = 0)
Model5<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff,
 iter_EM = 0)
Model6<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
```

```
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate +
 IOFGrate*TestNo,
 iter_EM = 0)
Model7<-mixed_model(CR ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ 1,
 iter_EM = 0)
Model8<-mixed_model(CR ~ TestNo + ObsNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo + PairAff*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo,
 iter_EM = 0)
Model9<-mixed_model(CR ~ TestNo + ObsNo +
 IOFGrate + PairAff,
```

```
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo,
 iter_EM = 0)
Model10<-mixed_model(CR ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = CR ~ TestNo +
 IOFGrate + PairAff +
 IOFGrate*TestNo,
 iter_EM = 0)
Model11<-mixed_model(CR ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary 1m Models:

```
Model1<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
```

```
 family = zi.poisson(),
 zi_fixed = Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 zi_random = ~1|ID,
 iter_EM = 0)
Model2<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 iter_EM = 0)
Model3<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ TestNo + ObsNo +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 iter_EM = 0)
Model4<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
```

```
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ TestNo + ObsNo +
 IOFGrate + IOFLine +
 IOFGrate*TestNo,
 iter_EM = 0)
Model5<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ TestNo + ObsNo +
 IOFGrate + IOFLine,
 iter_EM = 0)
Model6<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ ObsNo +
 IOFLine,
 iter_EM = 0)
Model7<-mixed_model(Flong ~ TestNo + ObsNo + PartnerEXP +
 IOFGrate + IOFLine +
```

```
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ 1,
 iter_EM = 0)
Model8<-mixed_model(Flong ~ TestNo + ObsNo +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ ObsNo +
 IOFLine,
 iter_EM = 0)
Model9<-mixed_model(Flong ~ TestNo +
 IOFGrate + IOFLine +
 IOFGrate*TestNo + IOFLine*TestNo,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ ObsNo +
 IOFLine,
 iter_EM = 0)
Model10<-mixed_model(Flong ~ TestNo +
 IOFGrate + IOFLine +
 IOFLine*TestNo,
 random = ~ 1|ID,
```

```
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ ObsNo +
 IOFLine,
 iter_EM = 0)
Model11<-mixed_model(Flong ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = Flong ~ ObsNo +
 IOFLine,
 iter_EM = 0)
Model12<-mixed_model(Flong ~ 1,
 random = ~ 1|ID,
 data = TOBPPT,
 family = zi.poisson(),
 zi_fixed = ~ 1,
 iter_EM = 0)
```

Supplementary Table 2. Experiment 2 Log likelihood ratio test results comparing model fit for general linear mixed-effects models and zero-inflated mixed-effects models.

Supplementary 2a. Social Salience Network								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ActivityonlyCondition	6	1554.26	1578.67	-771.13	1542.26			
ActivitynoRelation	11	211.96	256.71	-94.98	189.96	1352.297	5	< 2.2e-16
ActivitynoInt	13	202.4	255.29	-88.2	176.4	13.5593	2	0.001137
ActivitynoRegion	14	1556.99	1613.94	-764.49	1528.99	0	1	1
ActivitynoSide	19	62.16	139.46	-12.08	24.16	1504.824	5	< 2.2e-16
ActivityAll	20	63.89	145.26	-11.95	23.89	0.2668	1	0.605483
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	1555.02	1567.22	-774.51	1549.02			
ActivitynoSide	19	62.16	139.46	-12.08	24.16	1524.9	16	< 2.2e-16

Supplementary 2b. Whole Brain								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ActivityonlyCondition	6	47.928	61.588	-17.9638	35.928			
ActivitynoCnR	9	33.986	54.476	-7.993	15.986	19.9416	3	0.0001745
ActivitynoInt	10	32.345	55.112	-6.1727	12.345	3.6405	1	0.0563884
ActivitytwoInt	16	10.858	47.284	10.5711	-21.142	33.4877	6	8.45E-06
ActivitynoSide	22	9.456	59.542	17.2721	-34.544	13.402	6	0.0370776
ActivityAll	23	11.264	63.628	17.3678	-34.736	0.1913	1	0.6618327
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	68.361	75.191	-31.18	62.361			
ActivitynoSide	22	9.456	59.542	17.272	-34.544	96.905	19	1.94E-12

Supplementary 2c. Cerebellum								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ActivityonlyCondition	6	32.9	42.401	-10.4499	20.8998			
ActivitynoParAff	7	27.025	38.11	-6.5126	13.0251	7.8747	1	0.005013
ActivitynoInt	8	25.285	37.953	-4.6427	9.2853	3.7398	1	0.053132
ActivityoneInt	11	20.667	38.086	0.6665	-1.333	10.6183	3	0.013979
ActivityAll	14	22.092	44.261	2.9541	-5.9082	4.5752	3	0.20568
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	39.365	44.116	-16.6825	33.365			
ActivityoneInt	11	20.667	38.086	0.6665	-1.333	34.698	8	3.04E-05

Supplementary 2d. Periaqueductal Gray (PAG)								
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
ActivityonlyCondition	6	46.785	60.445	-17.392	34.785			
ActivityonlyJuvReun	7	40.998	56.934	-13.499	26.998	7.7871	1	0.005262
ActivitynoInt	12	36.005	63.325	-6.003	12.005	14.9922	5	0.010396
ActivityfourInt2	22	-9.24	40.846	26.62	-53.24	65.2455	10	3.64E-10
ActivityfourInt	24	-13.096	41.544	30.548	-61.096	7.8554	2	0.019689
ActivitynoSide	30	-8.48	59.82	34.24	-68.48	7.3841	6	0.286783
ActivityAll	31	-7.23	63.347	34.615	-69.23	0.7505	1	0.386323
	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Null1	3	67.299	74.129	-30.65	61.299			
ActivityfourInt	24	-13.096	41.544	30.548	-61.096	122.39	21	2.59E-16

Supplementary 2a Models:

ActivityonlyCondition: logTotalActivity ~ Condition + (1 | ID)

ActivitynoRelation: logTotalActivity ~ Condition + Region + (1 | ID)

ActivitynoInt: logTotalActivity ~ Condition + Region + JuvReun + ParAff + (1 |
ID)

ActivitynoRegion: logTotalActivity ~ Condition + JuvReun + ParAff + JuvReun * Condition +
ParAff * Condition + (1 | ID)

ActivitynoSide: logTotalActivity ~ Condition + Region + JuvReun + ParAff + JuvReun *
Condition + ParAff * Condition + (1 | ID)

ActivityAll: logTotalActivity ~ Condition + Side + Region + JuvReun + ParAff +
JuvReun * Condition + ParAff * Condition + (1 | ID)

Null1: logTotalActivity ~ 1 + (1 | ID)

Supplementary 2b Models:

ActivityonlyCondition: logTotalActivity ~ Condition + (1 | ID)

ActivitynoCnR: logTotalActivity ~ Condition + JuvReun + IOFProx + IOFLine +
(1 | ID)

ActivitynoInt: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFLine +
(1 | ID)

ActivitytwoInt: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFLine +
JuvReun * Condition + CnRPar * Condition + (1 | ID)

ActivitynoSide: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFLine +
JuvReun * Condition + CnRPar * Condition + IOFProx * Condition +
IOFLine * Condition + (1 | ID)

ActivityAll: logTotalActivity ~ Condition + Side + JuvReun + CnRPar + IOFProx +
IOFLine + JuvReun * Condition + CnRPar * Condition + IOFProx *
Condition + IOFLine * Condition + (1 | ID)

Null1: logTotalActivity ~ 1 + (1 | ID)

Supplementary 2c Models:

ActivityonlyCondition: logTotalActivity ~ Condition + (1 | ID)

ActivitynoParAff: logTotalActivity ~ Condition + JuvReun + (1 | ID)

ActivitynoInt: logTotalActivity ~ Condition + JuvReun + ParAff + (1 | ID)

ActivityoneInt: logTotalActivity ~ Condition + JuvReun + ParAff + JuvReun * Condition +
(1 | ID)

ActivityAll: logTotalActivity ~ Condition + JuvReun + ParAff + JuvReun * Condition +
ParAff * Condition + (1 | ID)

Supplementary 2d Models:

ActivityonlyCondition: logTotalActivity ~ Condition + (1 | ID)

ActivityonlyJuvReun: logTotalActivity ~ Condition + JuvReun + (1 | ID)

ActivitynoInt: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFGrate +
JuvVoc + ParAff + (1 | ID)

ActivityfourInt2: logTotalActivity ~ Condition + JuvReun + IOFGrate + JuvVoc +
ParAff + JuvReun * Condition + IOFGrate * Condition + JuvVoc *
Condition + ParAff * Condition + (1 | ID)

ActivityfourInt: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFGrate +
JuvVoc + ParAff + JuvReun * Condition + IOFGrate * Condition +
JuvVoc * Condition + ParAff * Condition + (1 | ID)

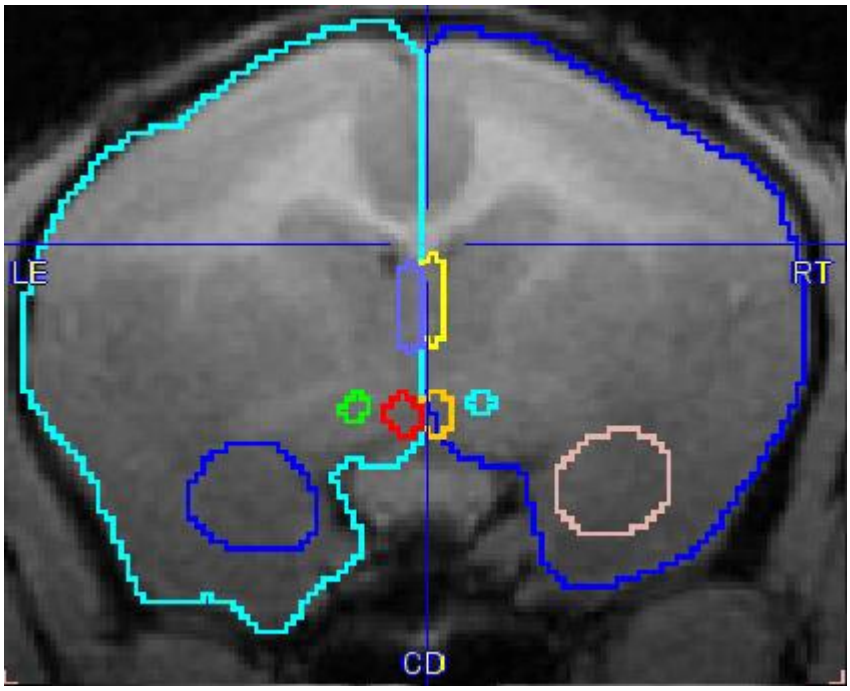
ActivitynoSide: logTotalActivity ~ Condition + JuvReun + CnRPar + IOFProx + IOFGrate +
JuvVoc + ParAff + JuvReun * Condition + CnRPar * Condition +
IOFProx * Condition + IOFGrate * Condition + JuvVoc * Condition +
ParAff * Condition + (1 | ID)

ActivityAll: logTotalActivity ~ Condition + Side + JuvReun + CnRPar + IOFProx +
IOFGrate + JuvVoc + ParAff + JuvReun * Condition + CnRPar *
Condition + IOFProx * Condition + IOFGrate * Condition +

JuvVoc * Condition + ParAff * Condition + (1 | ID)

Null1: logTotalActivity ~ 1 + (1 | ID)

Supplementary Figure 1. Example segmentation of regions of interest (ROIs). a) Magnetic resonance image (MRI) with segmentation of hypothalamus (left = orange; right = red), ventral pallidum (left = light blue; right = green), lateral septum (left = yellow; right = purple), and amygdala (left = pink; right = dark blue), left whole brain (dark blue outline around left hemisphere), and right whole brain (light blue outline around right hemisphere). b) Positron Emission Tomography (PET) image co-registered with MRI, including segmented ROIs. Warmer colors (e.g., yellow) indicate higher glucose uptake (see scale below co-registered image).

1a) 

1b)

