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Intranasal oxytocin does not change partner preference in female titi monkeys (*Plecturocebus cupreus*), but intranasal vasopressin decreases it

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Abstract

Strong social bonds are critical to human health, however, the mechanisms by which social bonds are formed and maintained are still being elucidated. The neurohormones oxytocin (OT) and vasopressin (AVP) are considered likely candidates. Primate females, both human and non-human, remain understudied populations. Here, we conducted a pharmacological study coupled with a behavioral partner preference test (PPT) to better understand the mechanistic basis of attachment in adult female titi monkeys (*Plecturocebus cupreus*). This pair-bonding species shares a conserved form of oxytocin with humans and is an excellent model organism to study the neural basis of social bonding. We performed intranasal administration of three doses of oxytocin (IN-OT), two doses of vasopressin (IN-AVP), one dose of an oxytocin antagonist (IN-OTA) and one dose of a saline treatment. We found that compared to the saline control, the IN-AVP treatment (lower dose, 40 IU/kg) decreased the time spent in proximity to the partner and increased lip-smacking toward the stranger. We found no effects of IN-OT or IN-OTA manipulation on partner preference. In contrast, low-dose IN-AVP weakened the partner preference in female titi monkeys.

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Contributions

Project design: KLB, SMF; Data collection and curation: PZT, LRW, MD, AW, AL, CK, JP, AB, AMHS; Data analysis: PZT, EF, KLB; Writing first draft: PZT; Writing and reviewing: PZT, KLB; Editing final version: All authors

Conflict of interest disclosure

The authors of the manuscript have no conflicts of interest to declare.

Ethics approval statement

All procedures and housing conditions were approved by the University of California Davis Institutional Animal Care and Use Committee.

Keywords

intranasal treatment; oxytocin; vasopressin; pair-bonding; females

Introduction

The formation and maintenance of close social relationships are of major importance for social species, including humans, as they play a role in the development and maintenance of good health. The neurological and hormonal basis of social behavior in mammals are under the strong influence of two neuropeptides, oxytocin (OT) and vasopressin (AVP) (Carter, 2017a, 2017b). Often termed as the “love hormone”, OT is involved in a large variety of social behaviors (Carter et al., 2020). While AVP is perhaps better known for its peripheral functions as an antidiuretic and vasoconstrictor, it is also highly implicated in social relationships (Winslow et al., 1993; Jarcho et al., 2011; Gouin et al., 2012). Both peptides have been shown to modulate a large spectrum of social behaviors including parental care, aggression, affiliation, and social recognition (Rigney et al., 2022). OT and AVP are notably associated with relationship quality (Plasencia et al., 2019), levels of affiliation (Jarcho et al., 2011; Bachner-Melman and Ebstein, 2014; Caldwell, 2017), interpersonal functioning (Gouin et al., 2012) and pair bonding (Bales et al., 2021).

Both OT and AVP are important for pair-bond formation in the monogamous prairie vole (*Microtus ochrogaster*) (Cho et al., 1999) as well as for pair-bond maintenance (Walum and Young, 2018; Berendzen et al., 2022). Pair bonding is a psychological construct based on attachment theory (Hazan and Shaver, 1987). Pair bonds include the hallmarks of attachment, such as preference for the partner, distress when separated from the partner, and stress buffering by the partner (Bales et al., 2021). However, how this integrated system works in primates is still unclear, and there are many species differences in the OT-AVP system (Freeman and Young, 2016). For example, OT receptors (OTR) and vasopressin V1a receptor (AVPR1a) distributions vary across primate species, with AVPR1a being generally more widespread, and OTR more restricted, than in rodent species (Freeman et al., 2014b, 2014a; Freeman and Young, 2016; Rogers Flattery et al., 2021).

Titi monkeys represent an excellent comparative model for studying the neurobiology of bonding because they have the capacity to form adult pair bond relationships (Bales et al., 2017). They also share a conserved form of oxytocin with humans (Lee et al., 2011). A large body of research has already been conducted in this species to better understand the neurological basis of pair bond formation (Fernandez-Duque et al., 2000; Fisher-Phelps et al., 2016; Bales et al., 2017; Hostetler et al., 2017; Arias del Razo et al., 2022b), but little work was carried out on long-term bonds (above 6 months), except for one recent study (Escriche Chova et al., 2023). Two studies have shown that both male (Maninger et al., 2017) and female (Zablocki-Thomas et al., 2023) titi monkeys in well-established pairs show behavioral reactions when confronted to a “jealousy”-provoking scenario, suggesting that, as in humans, long-term pair bonds in titi monkeys contain familiar emotional components.

There are relatively fewer studies on the role of AVP in primate affiliation than on the role of OT (Lu and Hu, 2021). The behavioral effects of AVP are frequently found to be opposite than those of OT (for instance, anxiogenic rather than anxiolytic), but both appear to be involved in adult attachment (Cho et al., 1999). For example, intracranial injections of AVP (but not OT) increased partner preference in male prairie voles (Winslow et al., 1993). In another study in male and female prairie voles from two different ecological backgrounds, 5 μg subcutaneous injections of AVP caused a stranger preference in some females, while none of a range of doses of AVP impacted stranger and partner preference in males (Cushing et al., 2001). Male titi monkeys treated with IN-AVP had increased contact with their pair mate and decreased contact with a female stranger presented in their home cage (Jarcho et al., 2011), supporting the hypothesis that AVP increases affiliative behavior toward the partner in some conditions.

Sex differences in the effects of OT and AVP are frequently found (Cushing et al., 2001; Lu and Hu, 2021), especially because of sex differences in the AVP system (De Vries and Panzica, 2006). It was initially thought that prairie vole males relied mostly on AVP for pair bonding (Winslow et al., 1993) and females primarily on OT (Insel and Hulihan, 1995), a view which has been reconsidered in the light of more recent studies (Cho et al., 1999; Berendzen et al., 2022; Duclot et al., 2022). In addition, OT and AVP activity are influenced by steroid hormones, with OT being estrogen-dependent, and some AVP neurons being androgen-dependent (Choleris et al., 2013; Aspesi and Choleris, 2022). These sex differences can help explain differences in the reactions of males and females to treatment with IN-OT (Arias del Razo et al., 2022a,b). For example, female (but not male) titi monkeys which received chronic treatment with IN-OT as periadolescents showed a blunted response to separation from their pair mate as adults (Arias del Razo 2022a); while adolescent IN-OT facilitated adult partner preference in males but not in females (Arias del Razo 2022b).

To address these sex differences, and to better understand the mechanistic basis of attachment in adult female primates, we performed a pharmacological study on the effects of OT and AVP on pair bond maintenance in female titi monkeys. We subsequently analyzed their preference for their partner relative to their preference for a male stranger. We chose to study females as there is still a gap in the literature of pharmacological and neuroscience studies for this sex (Woitowich et al., 2020; Zablocki-Thomas et al., 2022). IN-AVP treatments have been performed with adult male titi monkeys but not with adult females (Jarcho et al., 2011). In addition, given the physiological sex differences in reaction to OT and AVP treatments, a better knowledge of female biology is necessary to improve medical solutions for women's health.

Methods

Subjects and housing

Subjects were eight paired adult female titi monkeys (*Plecturocebus cupreus*) that had been with their partner for at least 6 months. These females were non-reproductive, either being housed with a vasectomized male or having received a tubal ligation (one subject). No hormonal contraception was used. All titi monkeys that participated in the study were born

in captivity at the California National Primate Research Center (CNPRC) and were housed indoors in 1.2x 1.2 x 2.1 m metal cages. Indoor temperature was maintained at 21°C. Lighting was on a 12h:12h light/dark cycle, with lights going on at 6:00 AM and off at 6:00 PM. Titi monkeys received a daily diet of monkey chow, carrots, bananas, apples, rice cereal, and *ad libitum* access to fresh water. They also received additional food enrichment twice a day in the form of two of the following items: spinach, apples, puffed cereals, oats, sweet peas, and sunflower seeds. All procedures and housing conditions were approved by the University of California Davis Institutional Animal Care and Use Committee.

Subject ranged in age from 3.08 to 6.16 years ($M = 4.59$, $SD = 1.06$) at the time of their first behavioral test. The time since they were paired (Pair Duration) ranged from 0.71 to 4.36 years ($M = 2.15$, $SD = 1.30$). For all subjects, their current partner was their first and only partner after they were removed from their natal family. All couples were considered as "long-term" pair bonded, as they were all pair-bonded for more than 6 months, and it was shown that titi monkeys have a strong preference for their partner by 4 months post-pairing (Arias del Razo et al., 2022b).

Pharmacological Treatments and doses

All subjects received seven treatments on separate testing days: three doses of oxytocin (0.8 IU/kg, 8.0 IU/kg and 80.0 IU/kg; Oxytocin acetate salt (5 mg), Santa Cruz Biotechnology), two doses of vasopressin (40 IU/kg and 80 IU/kg; [Arg8]-Vasopressin acetate salt = 95% (HPLC), Sigma-Aldrich Chemical Co Inc), one dose of oxytocin antagonist (OTA, 10 mg/kg, L-368,899, Tocris), and one dose of saline solution. We gave the treatments dissolved in saline in two deliveries of 45 ul each per nostril (for a total of 180 µl). Doses of OT were based on previous work in prairie voles and juvenile titi monkeys (Bales et al., 2007; Arias del Razo et al., 2020, 2022b, 2022a), and doses of AVP on a previous study in male titi monkeys (Jarcho et al., 2011). The testing days for each animal were spaced at least three days apart to ensure complete elimination of the previous treatment. Testing occurred approximately once a week, with a maximum interval of 22 days between tests.

Administration of OT and AVP

Titi monkeys are small-bodied primates (adults weigh on average 1.2 kg). Subjects were trained for intranasal dosing 2 weeks before the first behavioral experiment. This training included voluntarily entering a transport box, being hand caught by a handler, wrapped in a towel and receiving a peanut by the experimenter. For the last sessions of the habituation, they were also administered saline solution (approximately the same volume as in the test condition) with a pipette before receiving the peanut.

We prepared all the treatments to ensure that each subject received the appropriate dose based on their current body mass. We stored aliquots of the treatment solutions in microcentrifuge tubes at −80°C until use (Arias del Razo et al., 2020). We administered treatments before the behavioral test at approximately 9:00 am for all subjects (lights automatically turn on at 6:00 am). Treatments were defrosted and used immediately. One experimenter was holding the subject while another dripped the treatment with a pipette of 45 µl for each nostril, alternating nostrils. After each drip, the experimenter placed a finger

over the nostril for a few seconds to prevent the subject from sneezing out the compound, until the compound was absorbed (Arias del Razo et al., 2022a).

After the administration of a treatment, an uptake period of 30 minutes (Freeman et al., 2016) was observed during which the female subject and her partner were filmed in their home cage. After the 30 minutes of uptake, the subject and the stimulus males were released in the testing apparatus.

Partner Preference Test (PPT)

We used a PPT that has been validated for titi monkeys (Carp et al., 2016) to assess behavioral effects of our treatments. The test is a three-hour behavioral paradigm in which the subject chooses between maintaining proximity to their partner or to a stranger. The test is split into five 30-minute observation periods and a final catch-and-release session. This test measures partner preference through the proportion of time the subject prefers to stay in proximity to her partner or to another individual. The order of the seven treatments was counterbalanced, as was the location of the partner and the stranger (right or left). The subjects had a minimum of two days of rest between each test.

Apparatus—The testing chamber consisted of three adjacent cages (2.1 m × 1.2 m × 0.8 m), connected with each other by a wire mesh window (grate) next to the highest perch (30 cm × 30 cm, with a 1.3 cm × 1.3 cm square opening size). The grate allowed the subject to see or sit next to her pair mate or the stranger male without physical contact (Rothwell et al., 2020). The female subject was always placed in the middle cage and the stimulus males (pair mate and stranger) in the side cages.

Behavioral data collection—Data were collected live during five sessions of 30 minutes via focal animal sampling using Behavior Tracker software 1.5 (http://www.behaviortracker.com) with a predefined ethogram (Table 1). All observers were trained with an experienced observer until they reached 90% intra- and inter-observer reliability during live scoring.

Preference measure—Preference was measured by the proportion of total time that the test animal (1) spent in the preference zone of a stimulus male; (2) spent touching the grate on the window of a stimulus male and (3) the number of times the subject chose a stimulus male during the catch and release session. Other behaviors recorded included Aggression, Duetting, Chest Rub, Back Arch and Lip Smack (Table 1).

Catch and release session—After the 150 minutes of the test, a catch and release experiment was performed. During this session, the subject was boxed and removed from the apparatus before being released again in the testing chamber. An observer noted which preference zone the subject chose first over a maximum duration of five minutes. This was repeated five times and the preference selection was noted according to the ethogram (Table 1).

Choice of the stranger stimulus and cage side—Unrelated, stranger stimulus males were chosen to match the age range of the subject's partner (see Supplements) to avoid any

potential bias in attraction according to age. A total of 23 different stimulus males were used in this study and their behavior was recorded in case of abnormal behaviors. Each stimulus male was used in only one test for each female subject.

Contact Affiliation in the home cage

Affiliative behavior was recorded in scan samples daily (M-F) for all pairs from the colony as described in previous studies (Karaskiewicz et al., 2021; Witczak et al., 2022). Every other hour from 8:30 am to 4:30 pm, the distance between the two pairs is assessed as follows: Tail-Twining for sitting side-by-side with tails intertwined at least one turn (T), Contact for any bodily contact that does not include tail-twining (C), Proximity for sitting within arm's reach of one another (P), or None for none of the above (N). We retrieved information on affiliative behavior for the 180 days before the first behavioral test. We consider this affiliative behavior as a baseline to assess attachment between pair mates: the more affiliative behavior they display, the stronger the attachment. We calculated an averaged contact ratio as follow (just 'Affiliation' for the rest of the paper):

$$(Contact)\ Affiliation = \text{mean}(\frac{number\ of\ T + C\ events}{number\ of\ T + C + P + N})$$

Statistical analysis

For each PPT, we added the scores for each observation, so that the analysis was performed on the full 150 minutes of the test. We used a generalized linear mixed modeling (GLMM) with a 'quasibinomial' distribution for proportion data (glmmPQL function from MASS package) in order to account for overdispersion due to the small sample size, and with a 'quasipoisson' for count data (**See** Table 2).We ran backward stepwise regression model selection procedures for the outcome variables in Table 2. Treatment, Affiliation, and Pair Duration were used as fixed effects and the identity of the individual as a random effect. We selected the model with the lowest residuals and the fewest number of variables relative to the full model as the best representation of the data. We always retained Treatment in the final model because it was our main variable of interest in this study.

Results

1. Partner and stranger preference across treatments and effect of affiliation and pair age

1.1. Preference Zone Proportion—Over the 8400 minutes (140h) of testing, females spent approximately 72.4% of the time in proximity to a male altogether. The rest of the time was spent in the middle of the testing chamber, away from both males. They spent approximately 4314.1 hours in proximity to their mate and 1718.9 hours in proximity to the stranger, which represent 71.5% and 28.5% of the total social time respectively.

We found no effect of Pair Duration on Partner Proximity, and the best model included Affiliation and Treatment. The lower dose AVP treatment resulted in a decrease in partner preference as compared to saline (AVP 40 IU/kg, β=−0.74, SE=0.31, p=.023); while females in pairs with higher Affiliation showed less partner preference than pairs with lower Affiliation (Affiliation, β=−5.579, SE=2.038, p= .0339) (Figure 1A, Table S1.1).

For Stranger Proximity, the final model included only Treatment as a fixed effect. We found that the higher dose of AVP tended to increase preference for the stranger by increasing the proportion of time spent next to the stranger over total testing time (AVP 80 IU/kg, β=.724, *SE*=.368, *p*=.055) (Figure 1B, Table S2.2). The lower dose of AVT has a similar, but smaller effect (AVP 40 IU/kg, β=.633, SE=.371, p=.095). We did not find any effect of Treatment, Affiliation, and Pair Duration on Partner and Stranger Social Proximity (Tables S1.2 and S2.2).

For Total Social Proximity, no Treatments were significant, but Affiliation showed a significant decrease of Total Social Proximity for females in relationships with higher Affiliation (Affiliation, β=−8.321, *SE*=1.886, *p* =.0045) (Table S3).

1.2. Grate touching proportion according to treatment and effect pair duration and affiliation—We found no effects of Pair Duration, Affiliation or Treatment on Partner Grate Touching (Figure 2A, Table S4). There was a tendency toward lower Partner Grate Touching following treatment with high-dose AVP (80 IU/kg; β=−0.685, *SE*=0.361, *p*=.0649).

Treatment, Affiliation, and Pair Duration did not significantly affect Stranger Grate Touching (Figure 2B, Table S5) and Social Grate Touching (Table S6). Treatment, Affiliation, and Pair Duration did not significantly affect Partner and Stranger Social Grate Touching (Tables S4.1 and S5.1).

In our study, we had two pairs that were relatively new (Subjects 3 and 8), paired only 12 and 6 months before their first PPT, respectively. However, these two pairs were not the ones that spent more time in Social Proximity and in Proximity to their partner (Supplementary Figure S2); it was the two pairs that had the lowest Affiliation scores during home cage observations that spent the most time in proximity during the PPT.

2. Lip-smacking and aggression across treatment and effect of Pair Duration and Affiliation

We found no effect of Treatment, Pair Duration, or Affiliation on Partner Lip-smacking (Figure 3A, Table S7). For Stranger Lip-Smacking, our best model with only treatment showed that AVP (40 IU/kg) increased the frequency of Stranger Lip-Smacking as compared to saline treatment (β=1.569, *SE*=.483, *p*=.023) (Figure 3B, Table S8). A similar effect, but only as a tendency, was observed on the Total Frequency of Lip-Smacking to both males (40 IU/kg; β=.666, *SE*=.365, *p*=.075) (Table S9).

For Partner Aggression, our best fitting model with only treatment showed a significant effect of OT (0.8 IU/kg), which increased the frequency of aggression as compared to saline (β=1.098, *SE*=.49, *p*=.0297) (Figure 3C, Table S10). The frequency of Partner Aggression was very low overall. For Stranger Aggression, our final model showed that Treatment did not affect the frequency of aggression toward the stranger, Affiliation had a negative relationship with aggression (β=−15,38, *SE*=4.366, *p*=.0101) and Pair Duration had a positive relationship with aggression (β=1.0238, *SE*=.255, *p*=.0169). Females were more aggressive toward the stranger when they were in longer term, but less affiliative

relationships (Figure 3D, Table S11). For the Total Frequency of Aggression, the final model with only Treatment showed an effect of OTA (0.8 IU/kg), increasing the frequency of aggression as compared to saline (β=1,281, *SE*=.59, p=.034) (Table S12).

Duetting with the partner was significantly related to Pair Duration (β=0.843, *SE*=0.171, p=.004) and Pair Affiliation (β=−9.840, *SE*=2.472, p=.011) (Figure 3E, Table S13). OTA significantly increased Duetting with the Stranger (β=2.015, *SE*=0.761, p=.011), but this result was mainly driven by an outlier (Figure 3F, Table S14). Duetting with the stranger was rarely displayed by the individuals overall, with a few trial exceptions were duetting was particularly present. Finally, Duetting with both males was significantly related to Pair Duration (β=.668, *SE*=.207, p=.023) and Pair Affiliation (β=−8.887, *SE*=3.172, p=.038) (Table S15).

3. Number of Zone Crossing, Chest rubbing and Arching

For the total frequency of zone crossings, a model with only Treatment showed an increase of crossings for the highest dose of AVP (AVP 80 IU/kg: β=.409, *SE*=.184, p=.0310), as well as for the lowest dose of OT (OT 0.8 IU/kg: β=.399, *SE*=.184, p=.0357) as compared to saline (Figure 4A, Table S16). We found no effect of Pair duration or Pair Affiliation. Treatment did not affect the proportion of crossing in the Partner Preference zone nor in the Stranger Preference zone (Tables S17–S18).

We also found no effect of Treatment, Affiliation and Pair Duration on the occurrences of Back Arching and Chest Rubbing (Figure 4B–4C, Tables S19–S20).

4. Catch and Release session—We found no effect of Pair Duration, Pair Affiliation, or Treatment on the proportion of choices toward the partner during the catch and release session (Figure 5, Table S21). Over the 280 catch and release trials (56 PPT * 7 subjects), females chose their partner in 179 occurrences (=64%), the stranger in 76 (=27%) and made no choice until time out in 25 occurrences (=9%).

Discussion

The present study investigated the mechanisms underlying attachment in eight adult female titi monkeys (Plecturocebus cupreus), a species known for their pair-bonding behavior, by using IN treatment of two doses of AVP, three doses of OT, one dose of OTA and a saline treatment. By manipulating the levels of these neurohormones, we aimed to gain insights into their influence on attachment behavior. Our main findings are that low-dose IN-AVP weakened the partner preference in female titi monkeys and that IN-OT treatment at the doses used here did not alter partner preference.

Because sex differences in the effects of OT and AVP systems have been well described (Cushing et al., 2001; Dumais and Veenema, 2016; Lu and Hu, 2021), as well as differential behavioral responses, we strongly expected sex differences in the actions of OT and AVP compared to results in males from existing literature. For example, previous studies showed that male and female titi monkeys reacted differently to OT treatments as described below (Arias del Razo et al., 2020, 2022b), while in humans, AVP administration (IN-AVP in

humans), has differentiated effects on social interactions of men and women (Rilling et al., 2014).

A decrease in partner preference and an increase in stranger preference: effect of AVP

In our study, we found that IN-AVP treatment (40 IU/kg) decreased the time spent in proximity to the partner as compared the control condition. In addition, AVP (80 IU/kg) tended to increase the time spent in proximity to the stranger as compared the control condition. These results were also supported by a tendency for the subjects to touch their partners' grates less (AVP 80 IU/kg) and to lip smack more toward the stranger (AVP 40 IU/kg). Taken together, these results suggest that AVP reduces preference for the familiar partner in adult titi monkey females. This study also demonstrates a potentially dose-dependent effect of intranasal administration of AVP, with the lowest dose having a behavioral effect while the higher dose does not.

Because of a previous study in male titi monkeys, we expected that IN-AVP treatment in female titi monkeys would have an opposite effect than in male titi monkeys (Jarcho et al., 2011). The study in males previously found that in adult male titi monkeys, IN-AVP (80 IU/kg) increased the frequency of contact with their partner as compared to an empty cage and as compared to a stranger female (Jarcho et al., 2011). In adult prairie voles which received IN-AVP during early development (1 week of age), partner preference formation was impaired in males but not females after individuals reached sexual maturity (Simmons et al., 2017). Taken together, our results suggest that intranasal administration of AVP has distinct behavioral consequences in females compared to those previously reported in males. This difference may have significant social implications, as our findings indicate a lower proximity with the partner and a higher lip-smacking behavior for the stranger in the IN-AVP condition. These results suggest that we should anticipate different behavioral responses between males and females when considering IN-AVP as a medical intervention for individuals with social or behavioral disorders.

Little or no effect of OT and OTA

Neither OT nor the OT antagonist treatments significantly affected the different behavioral measures related to affiliation (Partner Proximity, Grate Touching, Lip-smacking), although the lowest dose of OT increased the number of zone changes and increased aggression toward the partner. However, this latter result was based on a very low frequency of aggression and seems to be mainly driven by an outlier; thus, it should be interpreted with precaution. In addition to promoting prosocial behaviors, oxytocin has also been suggested to enhance social salience by influencing both prosocial and antisocial behaviors (Shamay-Tsoory and Abu-Akel, 2016); for example, IN-OT can increase aggression in the context of provocation in humans (Ne'eman et al., 2016). As such, the social salience hypothesis could explain an increase in aggression from the females when they received OT.

Two other behaviors (Back Arching and Chest Rubbing), were also not present in many tests. Our results may suggest that titi monkey pair bonds are strong enough that OT treatments (both agonist and antagonist) probably do not affect an established bond, at least as far as affecting partner preference. It is unlikely that the observed results are

due to inadequate dosing. This is supported by the fact that a substantial amount of OT was administered intranasally, cited as 100 times the total pituitary content in our study. An alternative hypothesis could be that the treatment does not reach the brain. Mens et al" (1983) measured cerebrospinal fluid (CSF) levels in rats at various timepoints after delivering large doses of intravenous or subcutaneous OT and AVP, and calculated that about 0.002% of the administered dose entered the CSF (Mens et al., 1983). There has been no comparably detailed study since, but Leng and Ludwig (2016) analyzed seven studies that reported some measurements in CSF after IN-OT, and concluded that these indicated that at most 0.005% of the administered dose entered the CSF (Leng and Ludwig, 2016). However, we consider that a sufficient amount reaches the brain to have a behavioral impact, based on several pieces of evidence that will be discussed further.

Previous studies in titi monkeys have also found that IN treatments are behaviorally effective in some contexts (Jarcho et al., 2011; Arias del Razo et al., 2020, 2022b). For example, a study from our laboratory involved chronic intranasal OT treatments in subjects that received OT treatments as juveniles (Arias del Razo et al., 2020), and were part of a follow-up partner preference study as adults without receiving further treatments (Arias del Razo et al., 2022b). The chronic treatment consisted in daily administration of IN-OT (0.8 IU/kg), for six months starting at 12 months of age. As juveniles, OT treated females increased their preference for their parents as opposed to strangers, while males showed an increased preference for unfamiliar adults over their parents. It also led males to form pair bonds faster as adults, which was not the case in females, which represents additional evidence for sex differences in the effects of IN-OT. In our study, all females spent more time with the partner than the stranger overall (Supplements, Figure S1), as found in previous studies (Carp et al., 2016; Escriche Chova et al., 2023). We also found a significant effect of the smaller dose of AVP on Lip-Smacking toward the Stranger, and a significant effect of OTA on Duetting with the Stranger. However, both results were driven by two and one outlier respectively and these effects should be interpreted with caution.

Interestingly, our group found in a previous study that 4-months post-pairing, both control and OT-treated animals were spending significantly more time touching their partner's grate than the stranger's grate, in a PPT identical to the one used in the current study (Arias del Razo et al., 2022b), with no effect of the juvenile chronic IN-OT treatment. In our study, we did not find this grate-touching preference for the partner with our eight females (2.15 years post-pairing on average), which could suggest that females seek less contact as the pair becomes older (after increasing during the first months post-pairing). These findings converge with a recent study on the temporal changes in affiliation in titi monkey couples over their 60 first weeks of pairing, which showed that affiliation generally increases over the first 6 months of pairing and then decreases (Witczak et al., 2022). Our results seem to confirm this trend that seeking out physical contact (grate touching) shows a downward trend at this duration of pairing.

We found that females in more affiliative relationships prefer to spend less time engaging socially during the PPT and engage less in duetting with their partner, while females in lower quality relationships prefer to spend more time engaging socially during the PPT and engage more in duetting. This means that females spending more time in contact or in tail

twining with their partner in the home cage (over the 180 days before the experiment) are those who show less preference for their partner in the context of the PPT. However, our measure of home cage Affiliation is a result of dyadic behavior from both the male and female, and might not completely account for how much the female is attached to her mate. Indeed, a study shows that females are initiating proximity by approaching in 62% of the occurrences in the wild, suggesting that females are more involved in pair affiliation than males (Dolotovskaya et al., 2020). We also found that Partner Proximity Time and Total Social Time were repeatable within individuals across treatments (See supplements). Low but significant repeatability for Partner Proximity and for Total Social Proximity suggest that there is within-individual consistency in these variables, but that it has only a small effect. Other measures of relationship quality (such as separation distress and stress buffering) and other individual variables like temperament could play a role in this relationship and should be further investigated in the future.

A recent study has documented the effect of Affiliation (with a pair mate) and Pair Duration in several contexts with titi monkeys (Rothwell et al., 2020). The authors suggested that in newly-formed pairs, males and females both were proactive in the maintenance of a relationship (by initiating more proximity maintenance and aggressions toward strangers), while for long-term pairs, the male seemed to be the sex more involved in pair bond maintenance (i.e. showing more affiliative behavior, approaching more often), which is not necessarily true in the wild (Dolotovskaya et al., 2020).

Why would females in pairs with lower Affiliation be more social and more attracted to the partner than females in a more affiliative relationship? In humans, individuals (adult and infant) in insecure attachment relationships tend to try to reduce social distance with the attachment figure by being more “clingy”, and parent infant relationship during childhood also seem to explain features of romantic relationship in adulthood (Hazan and Shaver, 1987). This relationship is consistent with our finding in which females in lower quality relationships spent more time looking at their mate while separated for the PPT. It is possible that lower affiliation stems from a less secure attachment. In titi monkeys, father-daughter relationship quality can influence partner preference behavior in this kind of set up (Witczak, 2022).

Among the other effects that we found, the number of zone changes (considered as a proxy of locomotion in our study, as it measures location changes in the testing cage) was increased by the lowest dose of OT and the highest dose of AVP. Thus, IN-OT treatment may increase arousal in female titi monkeys. This is surprising as OT administrations generally shows an anxiolytic effect, by decreasing the release of adrenocorticotropin hormone and glucocorticoids (Legros et al., 1982; Neumann et al., 1999). However, OT and other neuropeptides often show U-shaped (or inverted U-shaped) dose-response curves in which some doses actually show opposite effects of others (Zhong et al., 2012).

Caveats and limitations—A common limitation related to primate experiments is relatively small sample size. In our case, we tested all the subjects available with our criteria at the time of the study (i.e. established pairs formed with non-reproductive females which were not receiving hormonal birth control). Nor were we able to test the role of an

AVP antagonist, due to our inability to find one that would enter solution sufficiently to administer intranasally. Finally, with the current design, we are unable to say conclusively whether the low dose of AVP acted through the AVP receptor itself, or through the OT receptor; a future study could use OTA as a blocking agent in conjunction with the low dose of AVP.

The negative result of OTA administration should also be approached with caution; although this drug has been shown to cross the blood-brain barrier (BBB, Boccia et al., 2007), and we have shown behavioral effects of this dose on juvenile female preference for her parents (Witczak et al., in review), it is still possible that a higher dose of OTA is necessary in order to block the majority of OT receptors.

The question of whether OT and AVP can cross the blood-brain barrier has been a topic of long and ongoing scientific inquiry, especially fifty years ago, where studies have reported contradictory data about BBB penetration. Some studies reported failures of intravenous injections and infusion of AVP to cross the BBB in dogs (Ang and Jenkins, 1982), while others reported that a small amount of peripherally delivered OT and AVP in rabbits could reach the central nervous system in approximately 10 minutes (Mens et al., 1983). More recent studies have also detected OT in the cerebrospinal fluid and different neural regions after intranasal administration in Rhesus macaques (Lee et al., 2020). A recent review concludes that OT delivery to the brain may result from different routes: direct penetration through intranasal administration and also crossing the BBB after traveling through the peripheral circulation (Yao and Kendrick, 2022). Intranasal treatments are a noninvasive method that allows nasal administration of pharmacological compound in a solution, which is meant to be delivered to the central nervous system through the olfactory and trigeminal nerves, ultimately crossing the BBB thanks to RAGE (receptor for advanced glycation end-products) receptors (Yamamoto and Higashida, 2020). Recent studies have found that RAGE transports OT across the BBB in sufficient quantity to affect behavior (Yamamoto et al., 2019), a process specific to IN (rather than intraperitoneal) administration (Potretzke et al., 2023). While it is likely that these treatments do penetrate the BBB, they also likely lead to high peripheral concentrations (Freeman et al., 2016). Peripheral effects of AVP, for example, would include higher blood pressure (Grzęda et al., 2023), and it is perhaps significant that the high dose of AVP increased locomotion in our study. While direct central administration would clarify this pathway, it was not feasible in our colony given the non-invasive focus of our primate research.

Conclusions and Future Research—We found that a low dose of IN-AVP decreased partner preference in adult females, while a low dose of IN-OT had an effect on aggression. We confirmed that low-dose AVP treatment has an opposite effect in female titi monkeys than previously found in males (Jarcho et al., 2011). This was the first study to test the effect of these treatments on a pair-bonded female primate, and thus has important insights for the neurobiology of social bonding in females. Other systems are important in attachment and pair bond formation in titi monkeys, but most of this work has also been performed only in males. Opioid receptors are also important for attachment in adult bonds (Ragen et al., 2015), as are serotonin and dopamine (Larke et al., 2016; Hostetler et al., 2017). Studying

these systems in females may reveal more sex differences that are critically important for women's health (Woitowich et al., 2020; Zablocki-Thomas et al., 2022).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

Data files are available in the supplementary files.

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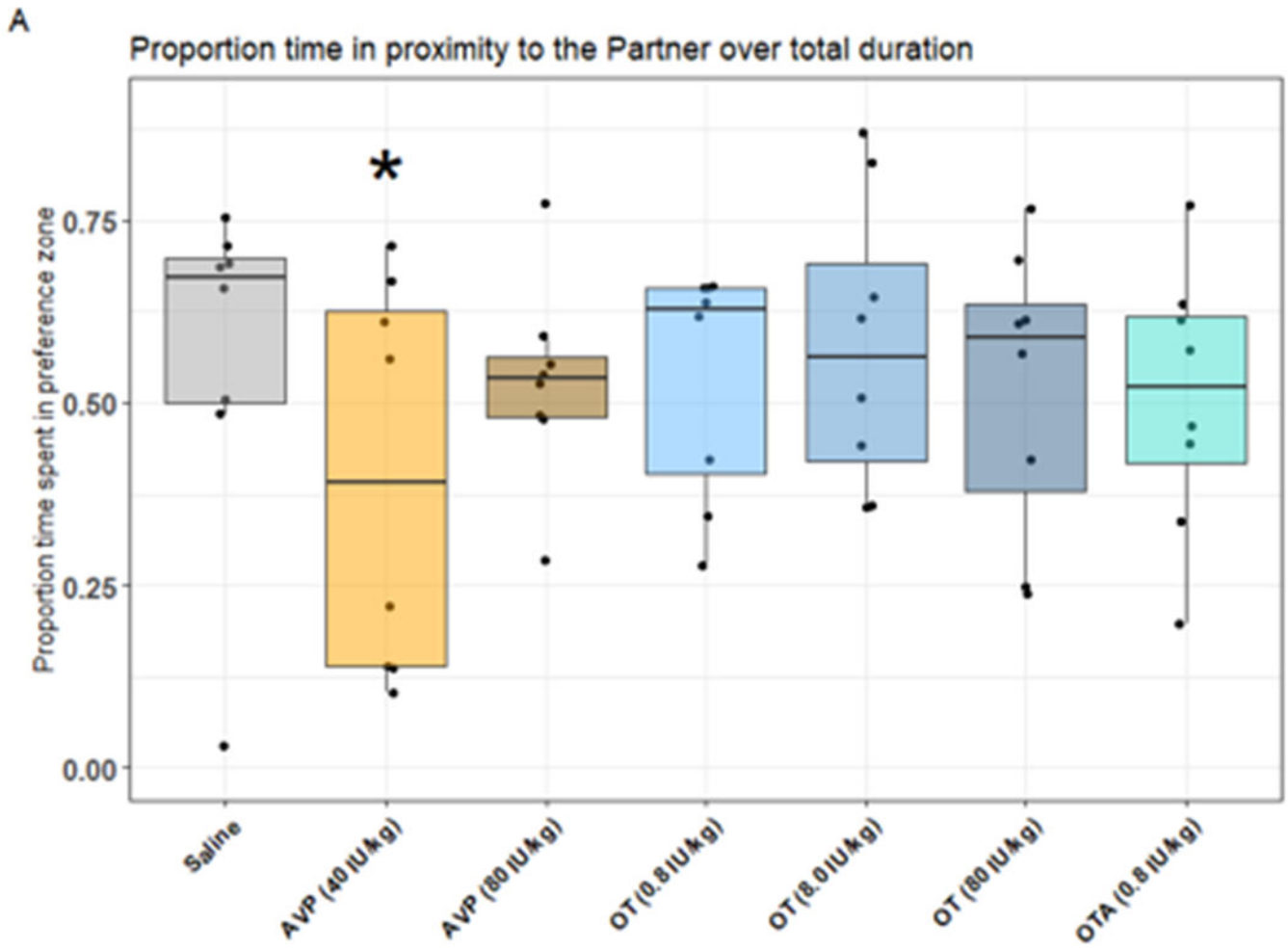
Highlights:

- We performed intranasal oxytocin (OT), vasopressin (AVP), and oxytocin antagonist treatments in pair-bonded adult female titi monkeys
- The lowest dose of AVP (40 IU/kg) decreased preference for the partner
- Locomotion was increased by AVP (80 IU/kg) and the lowest dose of OT (0.8 IU/kg).
- Females in more affiliative pairs showed lower preference and fewer duetting calls than females in less affiliative pairs



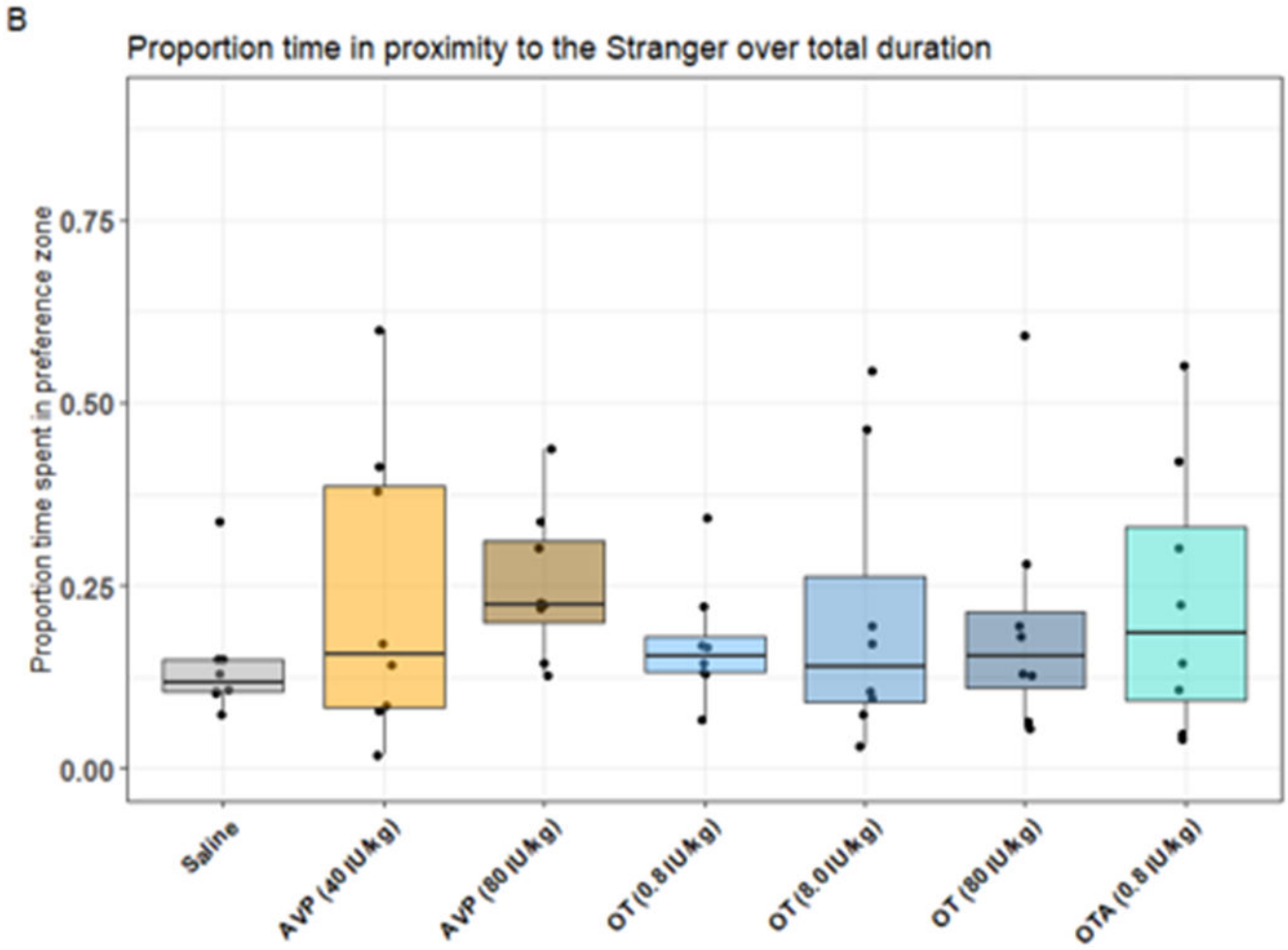
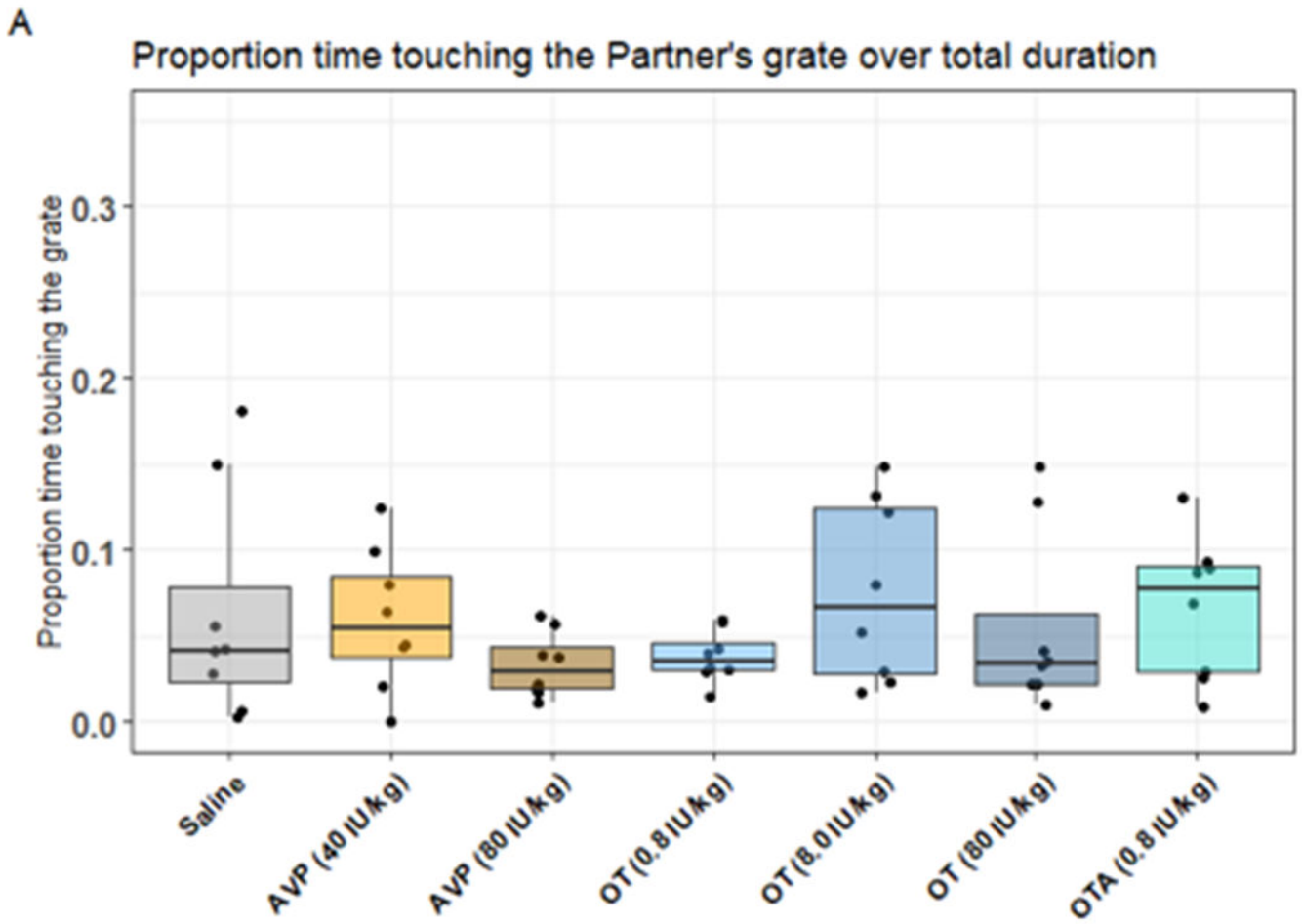


Figure 1:
Proportion of time spent in the partner zone (Partner Preference, panel A) or in the stranger zone (Stranger Preference, panel B) over the total duration of the test for the different pharmacological treatments.



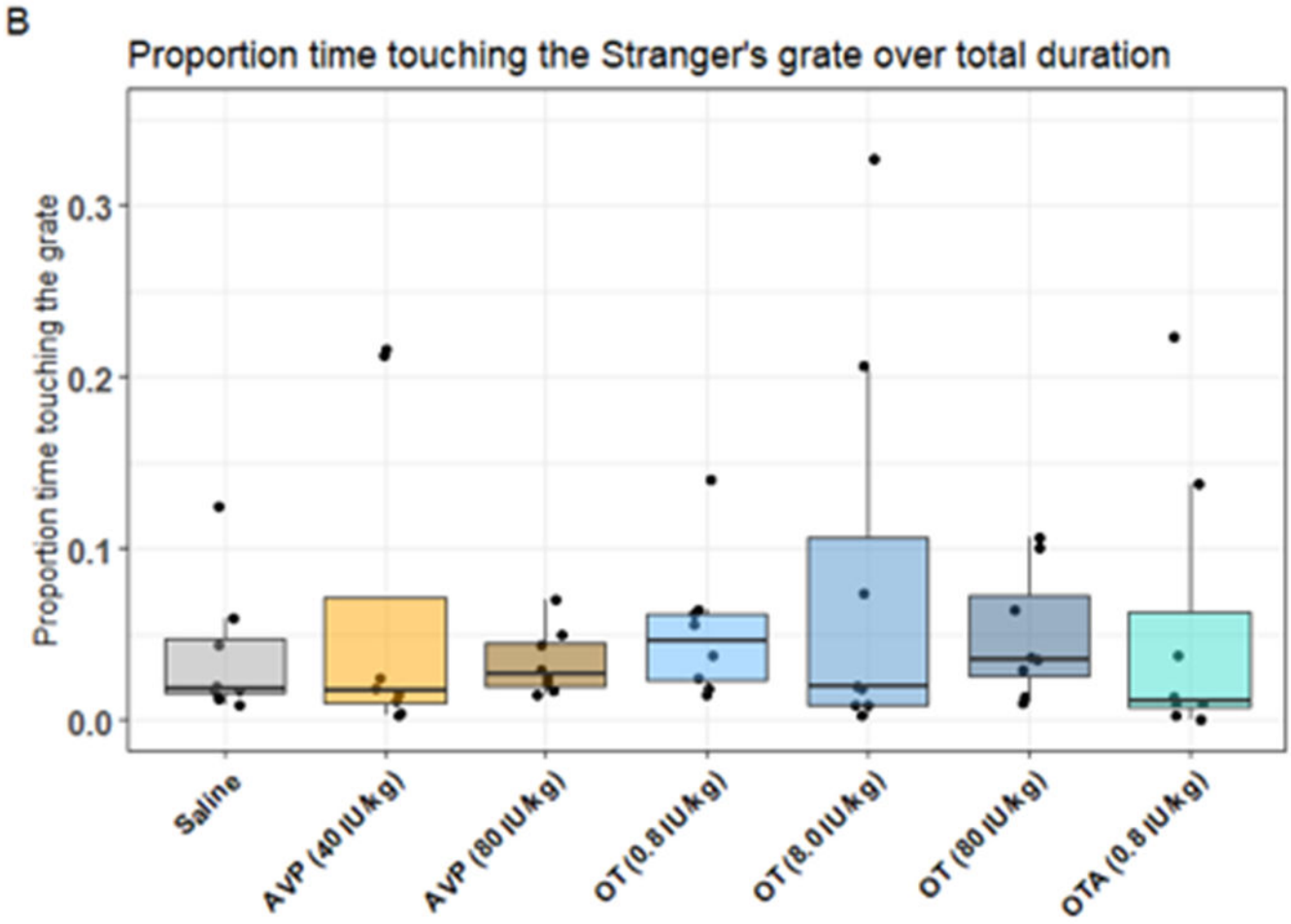
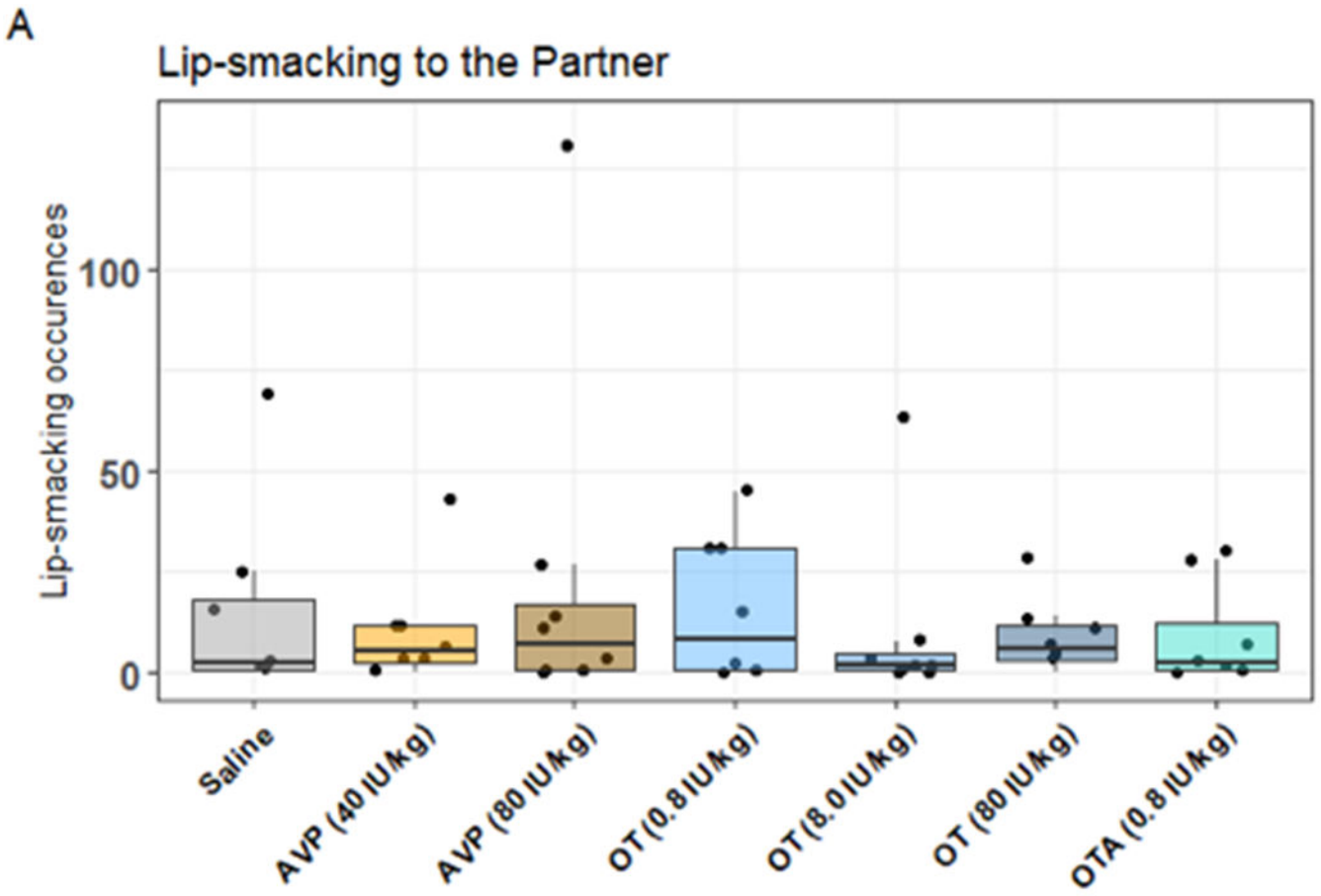
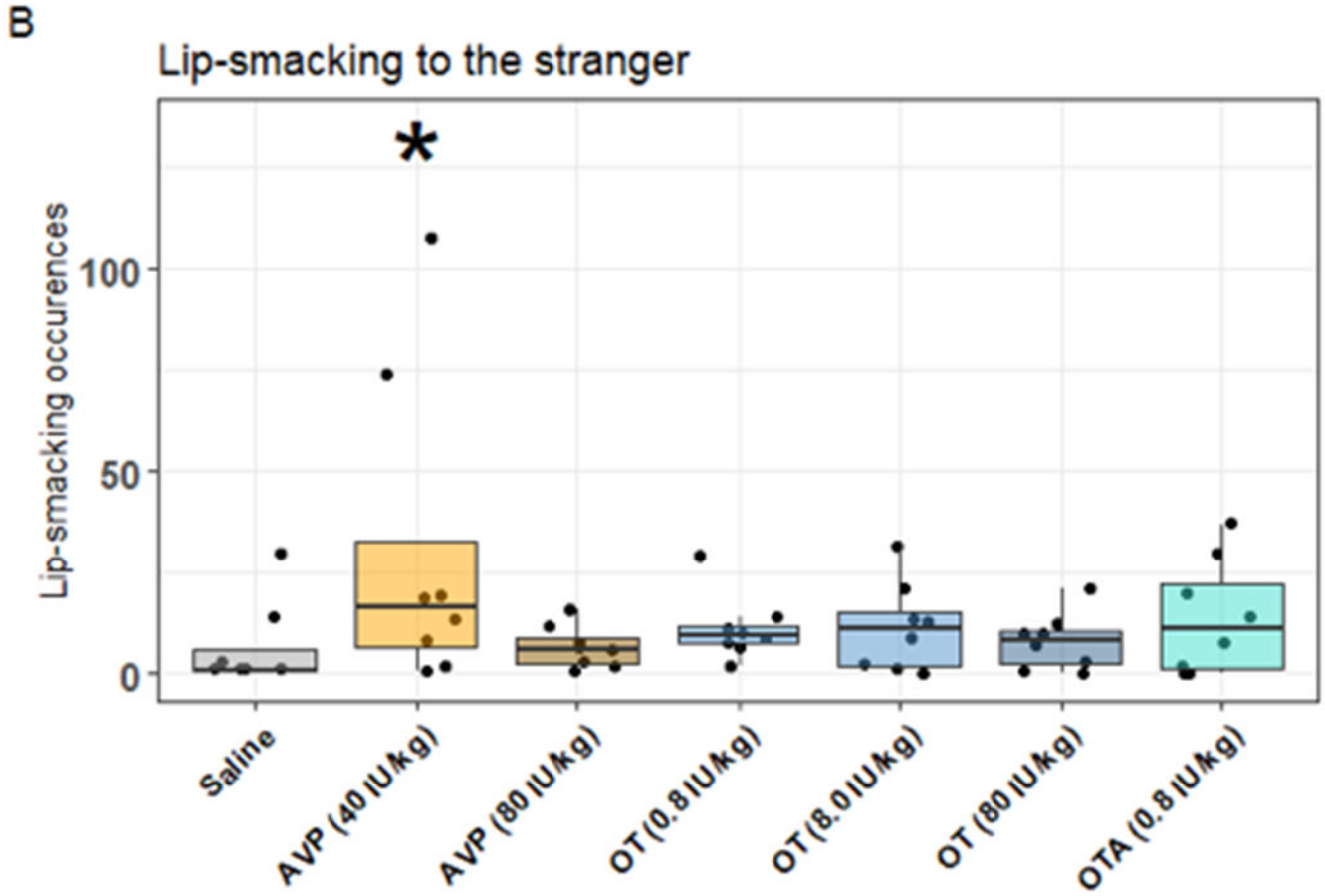
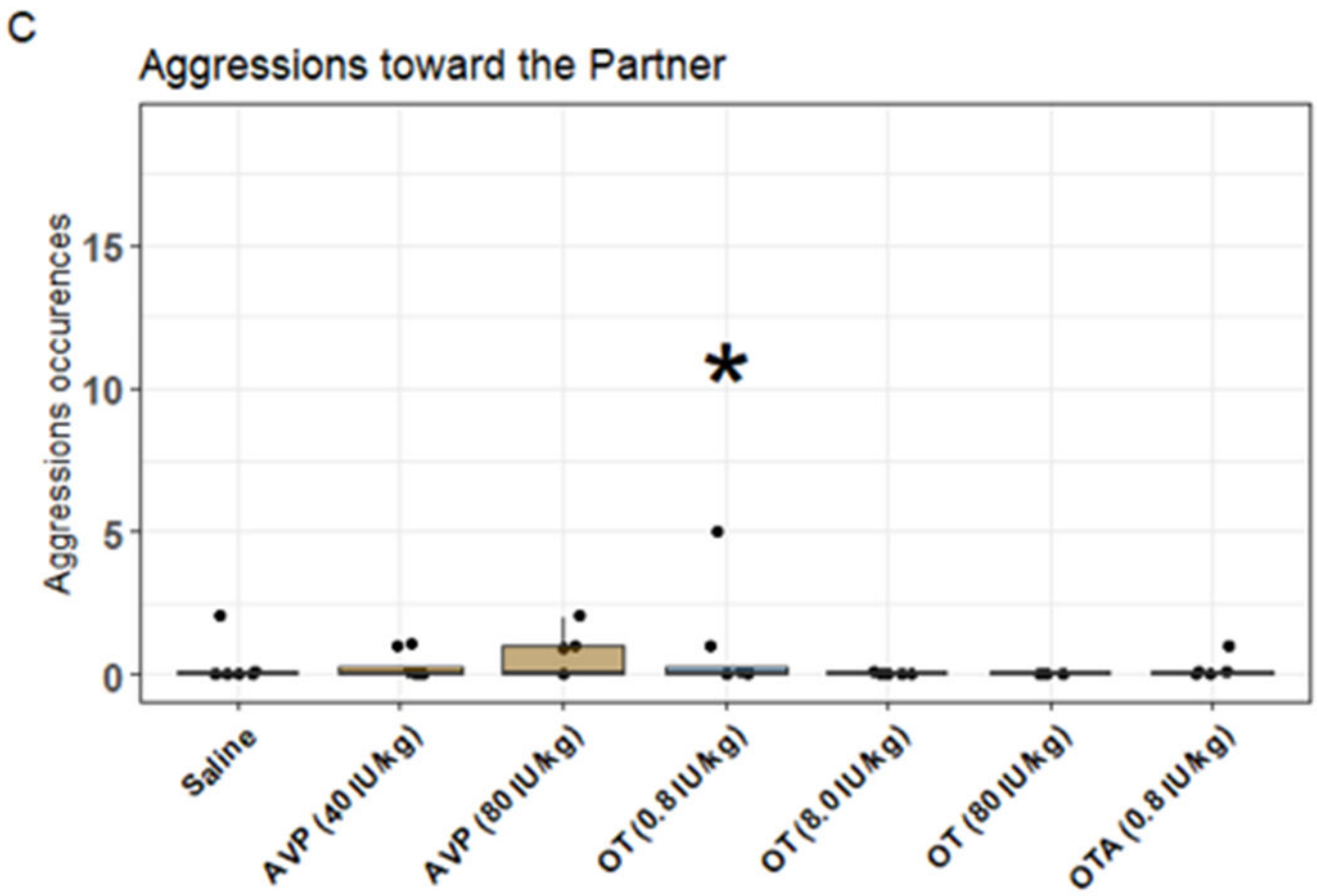
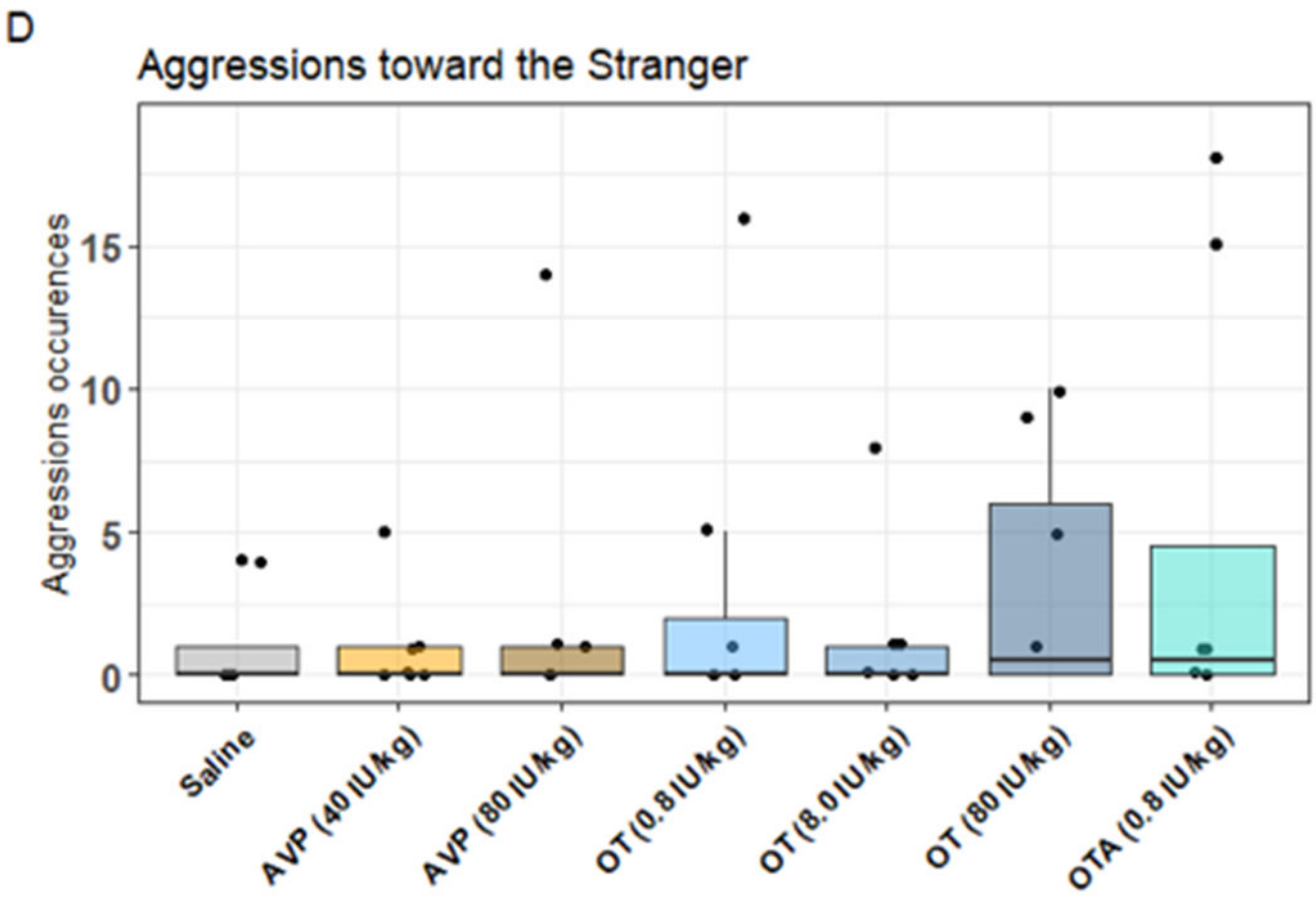


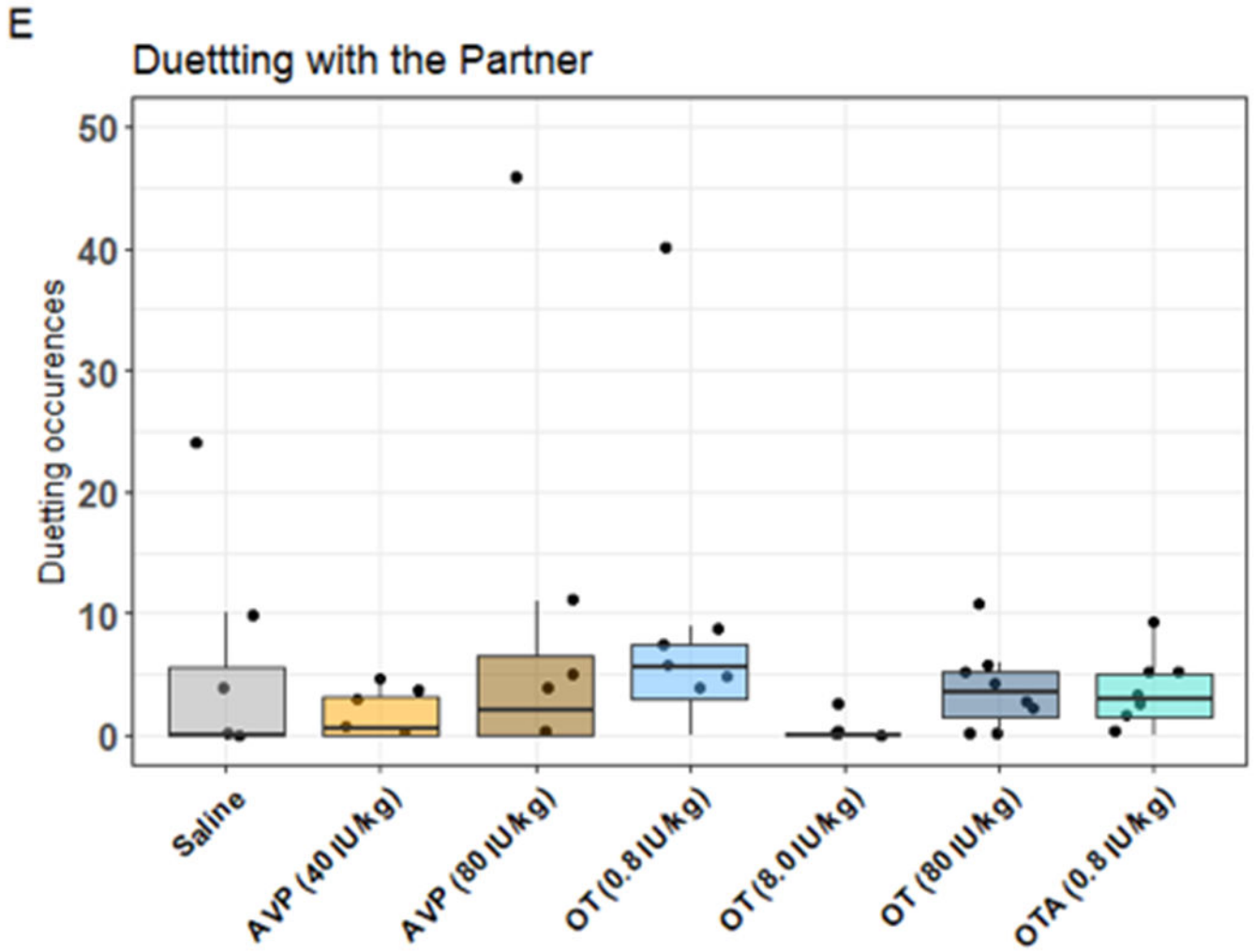
Figure 2:
Proportion of time spent touching the Partner's grate (Partner Grate Touching, A) or the Stranger's grate (Stranger Grate Touching B).











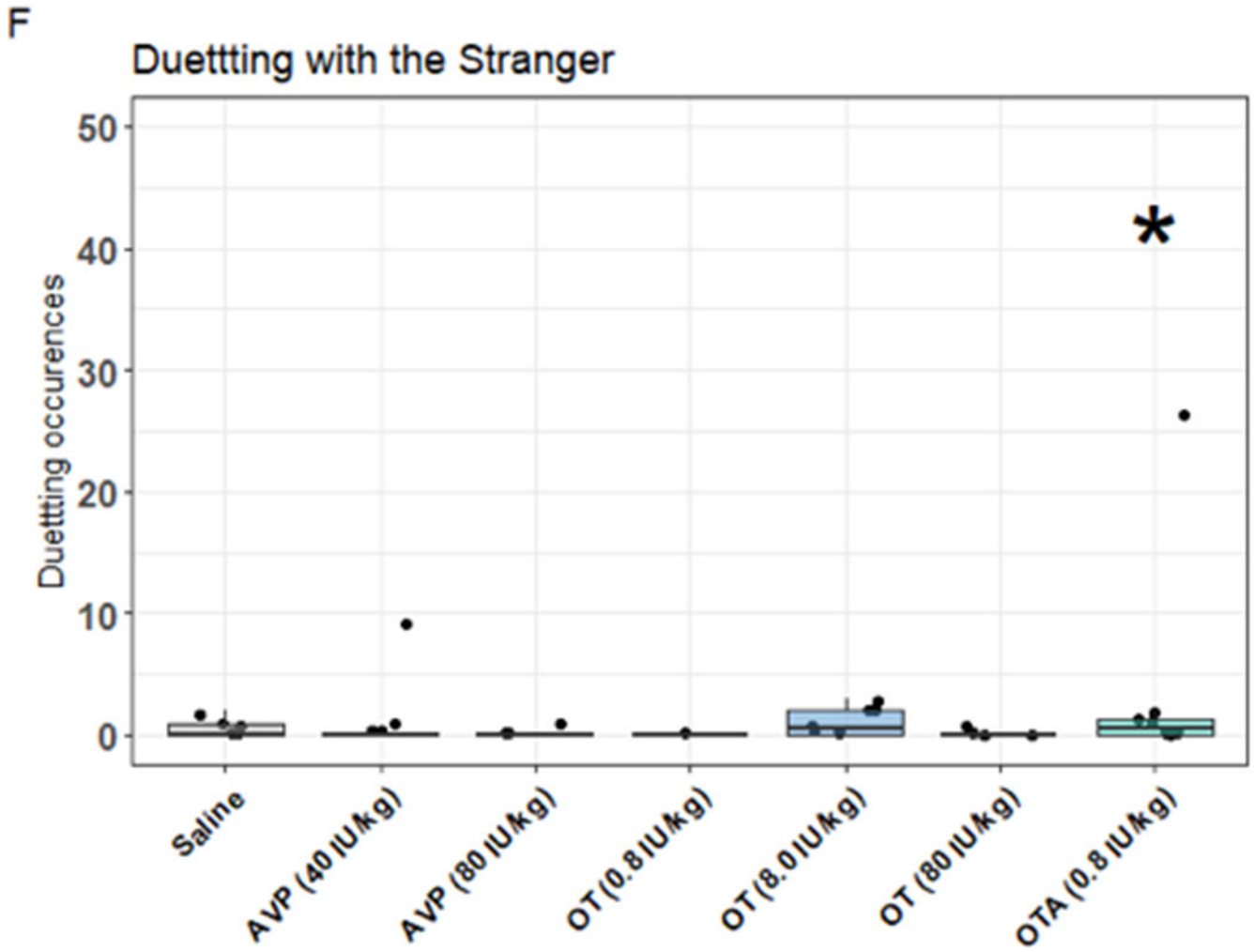
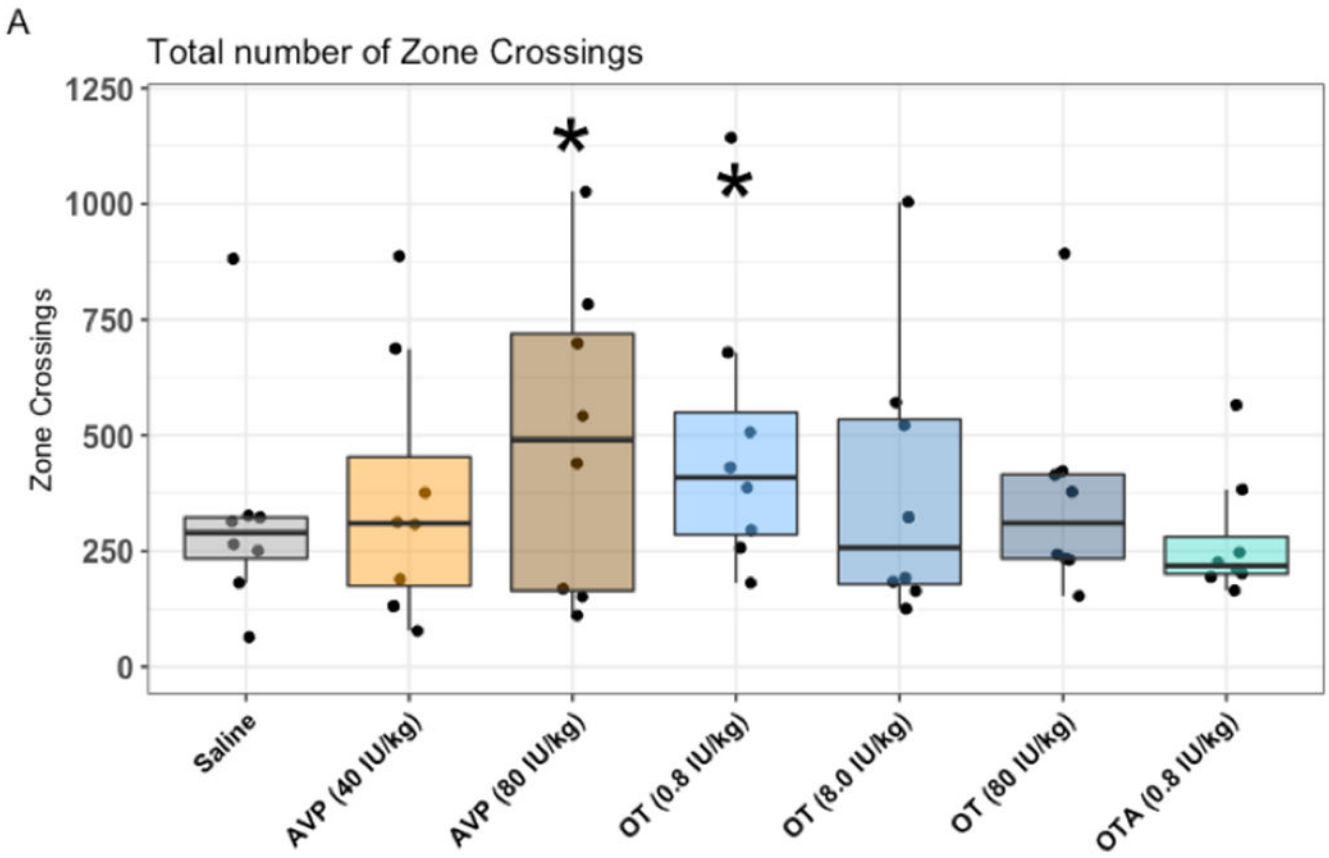
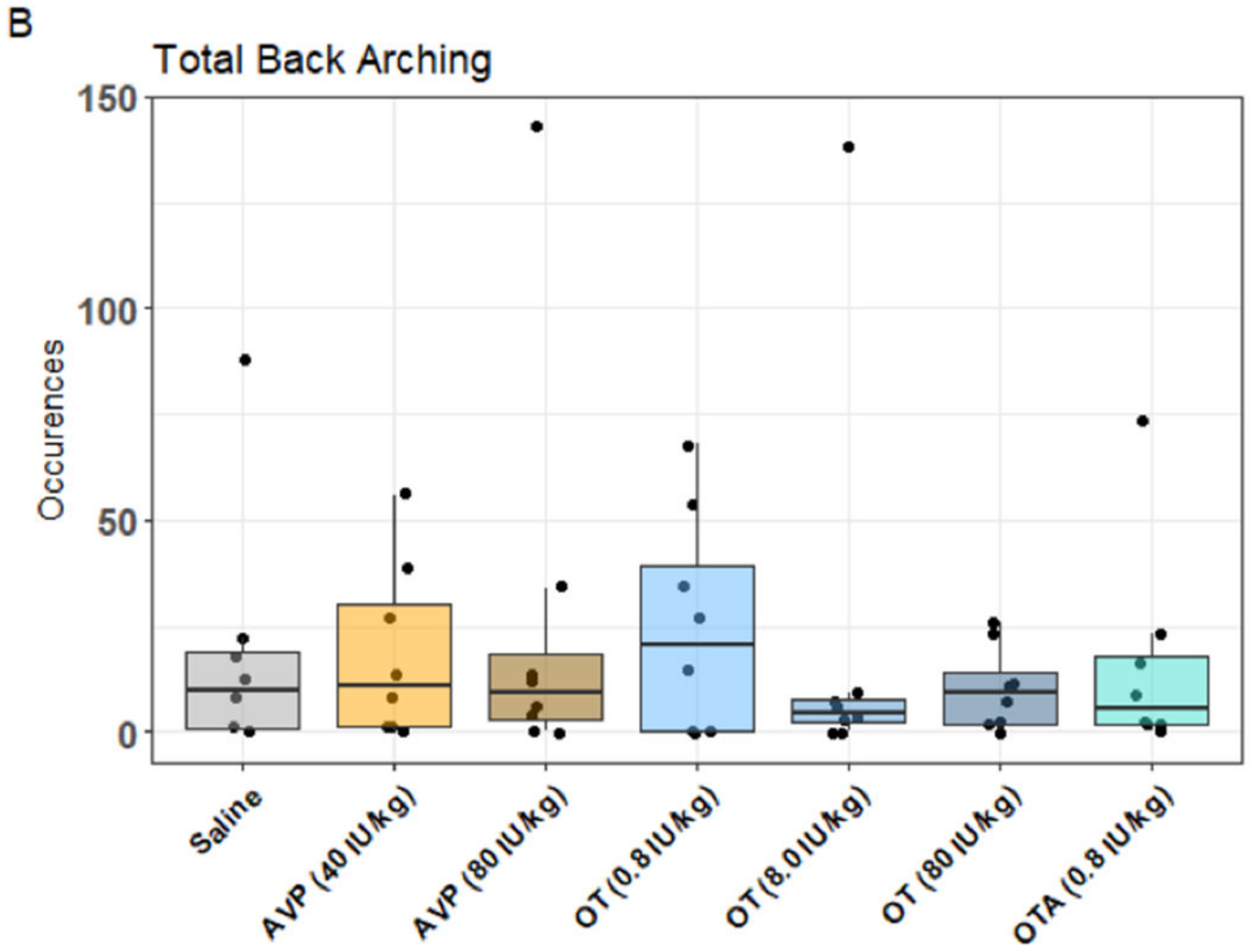


Figure 3:

Frequency of Lip-smacking towards the Partner (A), towards the Stranger (B), frequency of aggressions toward the Partner (C) and toward the Stranger (D), frequency of duetting with the Partner (E) and with the Stranger (F).





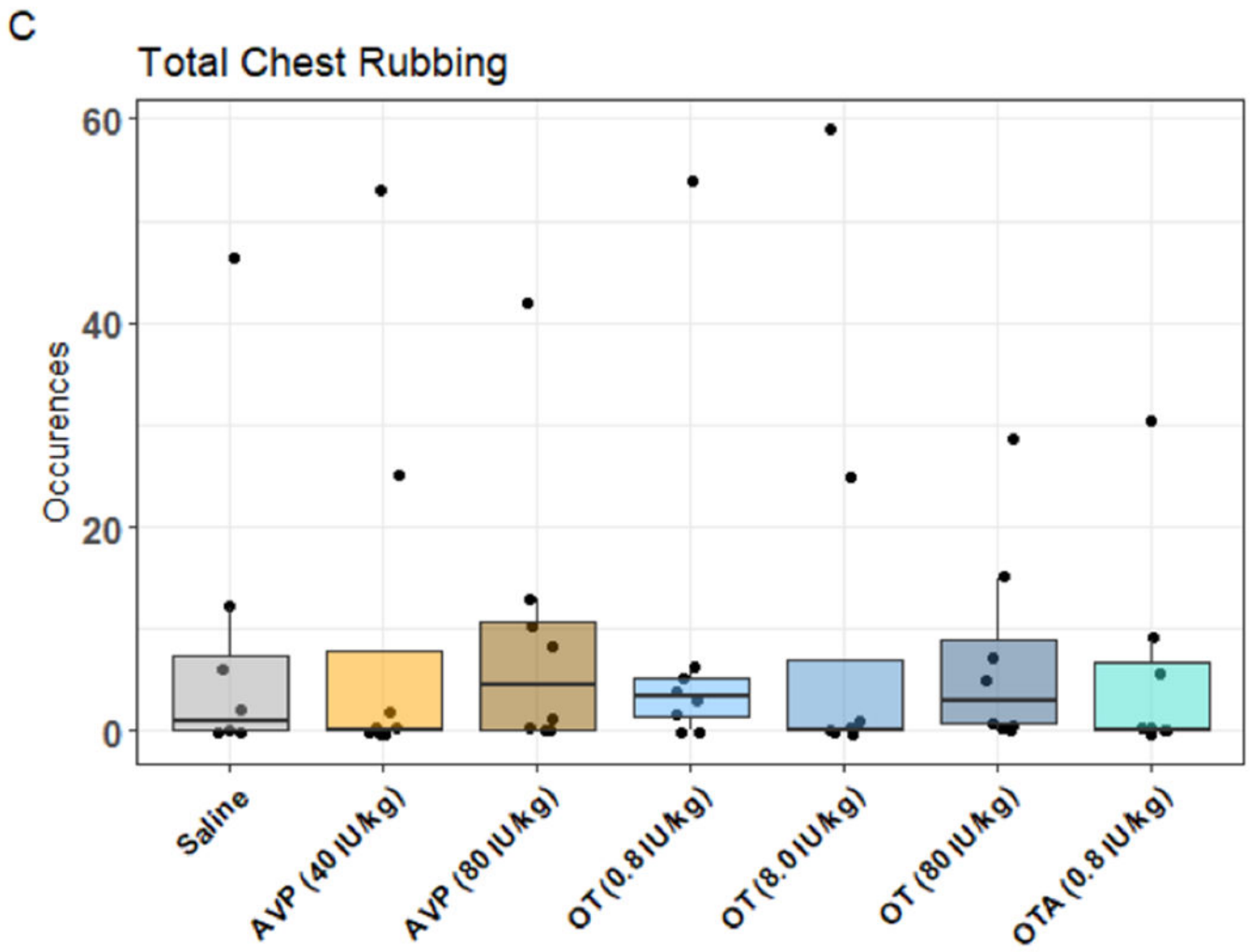


Figure 4:

Frequency of preference zone changes (Partner Preference zone, Stranger Preference zone or other) over the total duration of the test (A), frequency of Back Arching (B) and frequency of Chest Rubbing (C).

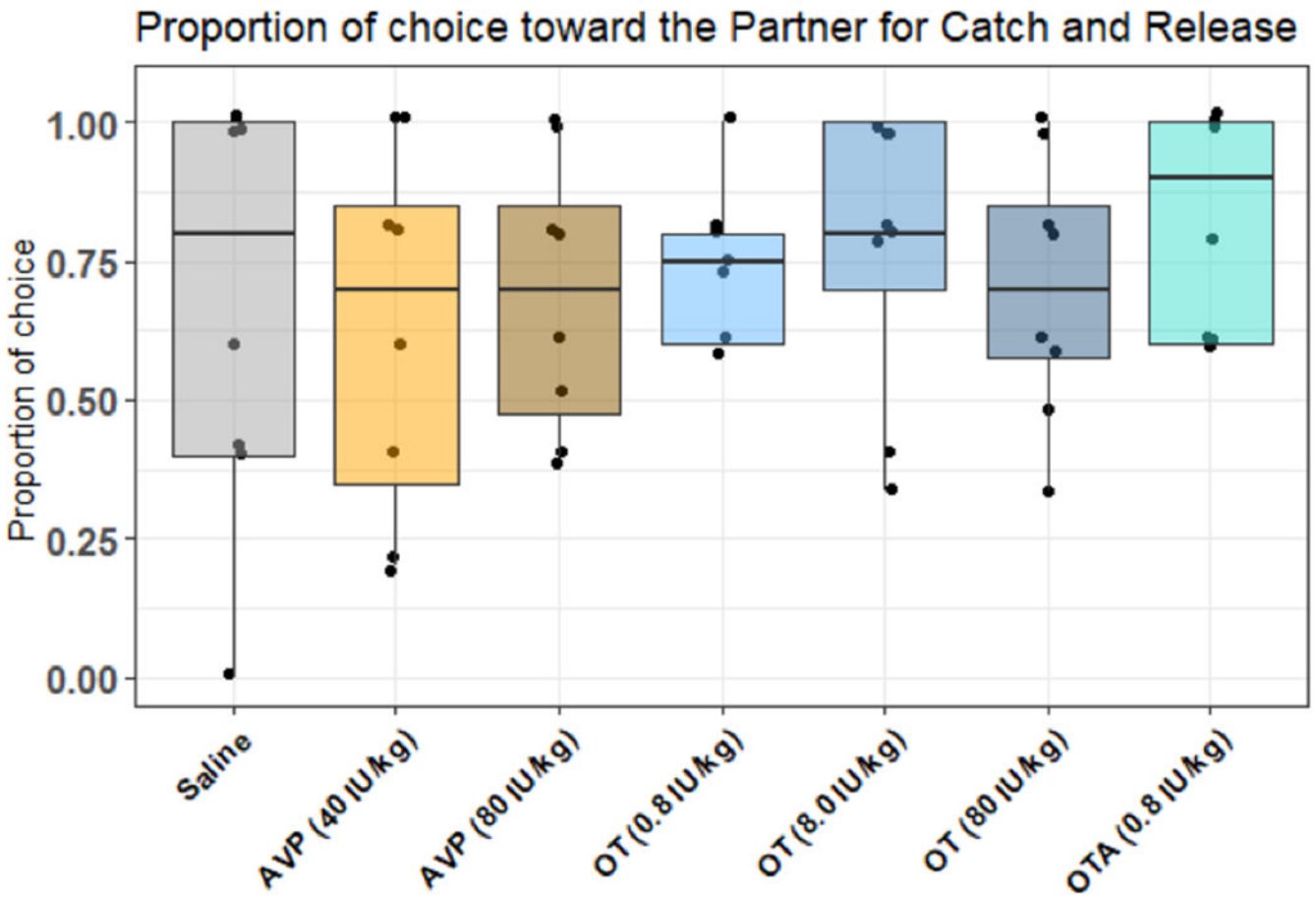


Figure 5:
Proportion of choice toward the Partner Preference zone during the Catch and Release session over the number of total decisions.

Table 1:

Partner Preference Test ethogram.

Observation sessions		
Name	**Type**	**Description**
Left/Right Preference Zone	Duration	Test animal enters the preference zone (with two or more limbs) near the left or right window.
Other Location	Duration	Test animal is located in any other part of the cage other than the preference zones (central area).
Left/Right Touch Grate	Duration	Test animal touches the grate on the left or right side of the test cage with any hands or feet for at least 1 second.
Left/Right Aggression	Count	Test animal displays by test animal that includes two of the following: (1) fast approach to stranger's grate, (2) forcefully touches the stranger's grate, (3) jumps on the stranger's grate, and (4) claws at the stranger's grate
Left/Right Lip Smack	Count	Test animal makes rapid lip movement accompanied by clicking/popping sound.
Left/Right Duetting	Count	Test animal begins long calling within 3 seconds of the stimulus pair on the Left/Right calling.
Back Arching	Count	Test animal raises dorsal surface of the back in an curving position AND/OR laterally whips the tail.
Chest Rubbing	Count	Test animal displays friction of the chest in a variety of forms. This may include scrubbing the chest with the hand or scrubbing the chest directly onto a perch or wall.
Zone crossing	Count	The test animal crosses the line from one location to another (preference zone or central area).
Catch and release session		
Preference Selection Left/ Right	Count	Test animal stays in the left or right preference zone for 10 consecutive seconds.
Time Out- No Choice Made	Count	The test animal fails to stay in either the left or the right preference zone for 10 consecutive seconds over a five-minute period.

Table 2:

Outcome variables analyzed in the generalized and linear mixed models.

Treatment of the variables:			
Name (distribution)	**Description**	**Meaning**	**Formula**
Total duration of the test (not a tested variable)	We summed for each test the total time scores as spent on the right preference zone, left preference zone and in the 'other location'.	Calculation of the accurate duration of the test (approx. 150 minutes but could slightly vary because of human errors during live scoring)	$time\ in\ left\ preference\ zone + time\ in\ right\ preference\ zone + time\ in\ Other\ Location$
Partner/ Stranger Proximity (quasibinomial)	We divided the time in a preference zone of the partner/ stranger by the 'Total duration of the test'.	The time spent in a preference zone reflects the choice from the subject to approach the male stimulus.	$\frac{time\ in\ partner\ or\ stranger\ preference\ zone}{total\ duration\ of\ the\ test}$
Partner/ Stranger Grate Touching (quasibinomial)	We divided the time in a touching a grate zone of the partner/ stranger by the 'Total duration of the test'.	Touching the grate may be an attempt to achieve physical contact (Rothwell et al., 2020)	$\frac{time\ touching\ grate\ of\ partner\ or\ stranger}{total\ duration\ of\ the\ test}$
Partner/ Stranger Social Proximity (quasibinomial)	We divided the time spent in the partner/ stranger preference zone by the total time spent in partner and stranger preference zone.	Over the total duration of approach to a stimulus, this represents how much of the total social time is spent in proximity to the partner or the stranger.	$\frac{time\ in\ partner/stranger\ preference\ zone}{time\ in\ partner + stranger\ preference\ zone}$
Partner/ StrangerSocial Grate Touching (quasibinomial)	We divided the time spent touching the partner's grate by the total time spent	Over the total duration of grate touching, this represents how much is directed towards the	$\frac{time\ touching\ partner/stranger\ grate}{time\ touching\ partner + stranger\ grate}$

Treatment of the variables:			
Name (distribution)	**Description**	**Meaning**	**Formula**
	touching either grate.	partner or the stranger.	
Total Social Proximity (quasibinomial)	We added the time spent in both preference zones and divided it by the total duration.	Over the total duration of the experiment, this represents the time during which the subject was in proximity to a male stimulus (including both the partner and the stranger).	$\frac{time\ in\ partner + stranger preference\ zone}{total\ duration\ of\ the\ test}$
Social Grate Touching (quasibinomial)	We added the time spent touching either grate and divided it by the total duration.	Over the total duration of the experiment, this represents the time during which the subject attempted to touch a grate.	$\frac{time\ touching\ partner + stranger\ grate}{total\ duration\ of\ the\ test}$
Lip-smacking (quasipoisson)	We counted the frequency of lip-smacking from the subject towards the partner or the stranger.	Lip-smacking is an affiliative behavior from the subject toward a stimulus.	*count of lipsmack toward partner, count of lipsmack toward stranger*
Aggression (quasipoisson)	We counted the frequency of aggression from the subject towards the partner or the stranger.	Aggression/ agonistic behavior (when the subject pounces on grated window)	*count of aggression toward partner*; *count of aggression toward stranger*
Duetting (quasipoisson)	We counted the number times the female joins a call from the partner or the stranger.	This is considered as an affiliative behavior.	*count of duetting with the partner, count of duetting with the stranger*

Treatment of the variables:			
Name (distribution)	**Description**	**Meaning**	**Formula**
Zone Crossing (quasipoisson)	We added the number of zone changes (partner preference zone, stranger preference zone, Other Location).	This is a proxy of locomotor behavior.	*Sum (count of entry in the Partner Preference zone + Stranger Preference zone + Other Location)*
Partner/ Stranger Zone Entry (quisaibinomial)	We counted the total number of entries in the Partner/ Stranger Preference zone and divided it by the total number of zone crossings.	Over the total number of zone changes, this assesses if the subject entered more often in the Partner or Stanger Preference zone.	$\frac{count\ of\ entry\ in\ the\ partner/stranger\ zone}{number\ of\ zone\ changes}$
Catch and Release Partner Preference (quisaibinomial)	For each trial, we counted the frequency that the female chose to go in the partner zone and divided it by the total number of selections.	Times out were not included. This choice represents the preference for the partner over the stranger.	$\frac{count\ of\ selection\ in\ the\ partner}{number\ of\ selections}$