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Neural and behavioral reactions to pairmates and strangers in monogamous female titi monkeys (*Plecturocebus cupreus)*

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Abstract

Pair bonding in humans and other socially monogamous species can have positive effects on health and well-being. These attachments also come with the potential for challenges such as separation, jealousy, or grief. Much of the work on the neurobiology of pair bonding in non-human primates has been carried out in coppery titi monkeys (*Plecturocebus cupreus*), a monogamous South American monkey, although these studies have been primarily in males. In the current study, we utilized female titi monkeys to experimentally examine responses to their monogamous male partner vs. a male stranger or being alone. Positron emission tomography (PET) scans were performed on eight adult female titi monkeys from well-established pairs. We used a within-subjects design in which each female underwent three different conditions after the fluorodeoxyglucose F^{18} (FDG) injection: a) the subject was reunited with her partner, b) encountered a stranger, or c) was alone in the experimental cage. Behavioural observations were recorded, and plasma assayed for cortisol. Females housed alone showed higher cortisol compared with either the partner or stranger conditions. FDG uptake was higher in the amygdala and hippocampus when interacting with the stranger than the partner. Proximity modulated the relationship between social condition and FDG uptake in several areas. Females entered into mutual proximity more frequently with the partner than with the stranger. Female titi monkeys have different physiological, neural, and behavioural reactions to being with their partner, a stranger male, or being alone.

Keywords

pair bond; monogamy; plasma cortisol; PET

Declarations of Interest

The authors have no conflicts of interest to report.

1. Introduction

Positive social attachments are essential for humans and many other social animals. Bonding facilitates survival and reproduction, helps to buffer against external and internal stressors, and enhances physiological and psychological well-being (Coria-Avila et al., 2014; Bales et al., 2017). Social disruptions, therefore, can induce severe health and psychological problems (Acevedo et al., 2020). In particular, social communication and bonding may be affected in conditions such as depression, schizophrenia, autism spectrum disorder, and anxiety disorders (Porcelli et al., 2019). For that reason, it is important to better understand these attachments and their underlying neural mechanisms.

In humans, adult social attachments are usually studied as romantic love between two partners. “Romantic love” is commonly viewed as a natural addiction (Burkett & Young, 2012; Fisher et al., 2016) that has evolved into a complex behavioural, neural, and physiological construct. Studies point toward specific patterns of neural activation that accompany the experience of romantic love (reviewed in Zablocki-Thomas et al., 2022). Specifically, the experience of passionate love in humans is associated with activity in reward-related brain areas associated with motivation (Stein & Vythilingum, 2009; Ueda & Abe, 2021). During fMRI studies when subjects were shown a picture or the name of their loved ones, researchers observed activation of brain areas such as the nucleus accumbens (NAcc), caudate, putamen, anterior cingulate cortex (ACC), and deactivation in brain areas such as the amygdala (Ortigue et al., 2010; Stein & Vythilingum, 2009). Studies also suggest that the nonapeptides oxytocin (OT) and arginine vasopressin (AVP), in addition to dopamine-rich regions, play an important role in romantic love and its maintenance in humans as well as in other monogamous animals (Acevedo et al., 2012; Walum & Young, 2018; Acevedo et al., 2020).

Moreover, the loss or separation from the partner is associated with a suite of psychological, neurobiological and hormonal changes, including depression and anxiety symptoms (Gündel et al., 2003; Shear, 2015), dysregulation of the HPA axis including higher levels of cortisol (Irwin et al., 1988; Ong et al., 2011; O’Connor, 2019), and activation of the amygdala (Ortigue et al., 2010). Romantic love, therefore, is a highly rewarding experience that can lead to physical and behavioural pathologies when disrupted. Despite the knowledge acquired from human research, the limitation of human experimentation leaves us with the need to use a suitable animal model to further investigate the neurobiology underlying romantic love, or “adult attachment”.

One of the most extensively studied models for adult attachment is the prairie vole *(Microtus ochrogaster*), a socially monogamous rodent. Prairie voles develop strong pair bonds with their partners, resulting in behavioural, neural, and physiological changes (Young, 2003; McGraw & Young, 2010; Young et al., 2011; Johnson & Young, 2015). In prairie voles, disruption of social bonds can also manifest in stress-related diseases, disorders, and behaviours (McNeal et al., 2014; Grippo et al., 2021). The presence of the well-established partner acts as a social buffer, a phenomenon in which a partner can reduce the response to external stressors (Lieberwirth & Wang, 2016; Kiyokawa & Hennessy, 2018). However, the study of pair bonding using a rodent model comes along with the knowledge that their

neurobiology differs significantly from human biology, potentially reducing translational potential, and suggesting that a non-human primate model may be of use (Phillips et al, 2014). Some of these differences are specific to the “social brain” and the OT system. For instance, in the rodent brain, the OT receptor (OTR) is often densely distributed in the olfactory areas while in primates, OTR are more commonly found in brain areas responsible for visual processing (Freeman & Young, 2016). While in both prairie voles and pair-bonding primates there is overlap between OTR and dopaminergic areas of the brain, the specific areas differ [the nucleus accumbens (NAcc) in prairie voles and the lateral septum (LS) in titi monkeys: Insel & Shapiro, 1992; Freeman et al., 2014].

Given the differences in the OT system between rodents and primates, studying social attachments in a closer evolutionary relative could provide valuable translational insights. We have previously demonstrated the benefits of using socially monogamous titi monkeys (*Plecturocebus cupreus*), for studying the neurobiology of social attachments (Bales et al., 2017). Titi monkeys, like prairie voles, establish pair bonds that are demonstrated by a preference for their partners over strangers, which is expressed by maintaining proximity to their partner (Carp et al., 2016). They show more affiliative behaviours towards their partner (Carp et al., 2016) and distress in response to involuntary separation (Ragen et al., 2012; Hinde et al., 2016). Finally, partners, but not other individuals, act as a social buffer that reduces the stress response (Mason and Mendoza, 1998). The formation and maintenance of a pair bond results in long-term neural changes (Bales et al., 2007; Hinde et al., 2016; Maninger et al., 2017a) in male titi monkeys. While previous experimental studies on the neurobiology of pair bonds in titi monkeys have been primarily performed in males, less is known about the neurobiology of female pair bonding.

The purpose of the current study was to investigate the behaviour and neurobiology of social attachment in female titi monkeys with a well-established partner, in the context of other aspects of pair bonding that follow bond formation (such as separation distress, rejection of alternative partners, and social buffering). Our overall hypothesis was that female titi monkeys utilize their pair bond relationship with their partner to self-regulate in a novel situation. Correlates of this hypothesis were that: a) females would show higher affiliative behaviour towards their partners over strangers, and spend more time in proximity and mutual proximity to their partners (Carp et al., 2016); b) their partner (and not a stranger male) would buffer their physiological reactivity to stress during a novel stressful situation; and finally, c) female neurobiology would reflect a separation response in the alone condition, a novelty response in the stranger condition, and an attachment response in the partner condition. Specifically, we predicted that females in the partner condition would have higher FDG uptake in regions of interest (ROI) identified as either “reward” areas (nucleus accumbens, caudate, putamen and anterior cingulate cortex), areas that produce OT and AVP such as the paraventricular and supraoptic nuclei of the hypothalamus (PVN and SON), social areas of the brain such as the lateral septum (LS), and areas related to social memory such as the hippocampus (Maninger et al., 2017a; Bales et al., 2017). During the alone and stranger conditions, we predicted higher FDG uptake in areas such as the amygdala, and higher plasma cortisol, because of the separation response (absence of the partner), and specificity of the partner as a social buffer (Ragen et al., 2013). We expected that the proximity of both animals in the partner and stranger condition would influence both

neural and hormonal outcomes, for instance, proximity of the partner reducing cortisol while proximity of the stranger would increase cortisol or have no effect.

Our outcome measures included neural glucose uptake as measured by PET scans with FDG; plasma cortisol, and behaviour in three different conditions in which the female was alone, with her well-established partner, or with a stranger male. Plasma cortisol can reflect metabolic needs rather than stress (for example pregnancy: Bales et al., 2005), however, it is a well-validated measure of psychological stress in titi monkeys (Arias del Razo et al., 2022b; Hinde et al., 2016; Hoffman et al., 1995; Mendoza & Mason, 1986).

2. Methods

2.1. Housing and animal care

All animals were part of the California National Primate Research Center (CNPRC) titi monkey colony. Titi monkeys were housed indoors at the CNPRC, in 1.2m x 1.2 m x 2.1 m cages with 4 perches positioned around the inside home cage, food bowl, and two water dispensers. The subjects were kept on a 12:12h light dark cycle and rooms were maintained at 21°C. Subjects were housed only with their partners. Food was provided twice a day (at 8:30h and 13:30h), and water was provided ad libitum; for more details about husbandry see (Mendoza, 1999; Tardif et al., 2006). All housing conditions and experimental procedures described were approved by the University of California Davis Institutional Animal Care and Use Committee.

2.2. Subjects

Test subjects were eight captive female adult titi monkeys with well-established partners. The male partners of the female subjects were used during the behavioural observations and PET scan procedure. Subjects had been paired with their current mate ranging from five months up to six years prior to the beginning of the study (Table 1). In previous studies, we have found that both male and female titi monkeys exhibit robust partner preferences by four months post-pairing (Arias del Razo et al., 2022a). The study used a within-subjects design in which all adult females received all three experimental conditions: alone condition, partner condition and stranger condition. The conditions were counter balanced. Baseline cortisol was also collected prior to the study in their home cage.

2.3. Procedures

2.3.1. PET Scan Procedure and Blood Sampling—The PET scan was performed at the CNPRC as in previous studies (Bales et al., 2007; Maninger et al., 2017a,b). Test animals undergoing PET imaging were moved to a smaller cage in a different building together with their partners. Due to their sensitivity to novel environments (Hennessy et al., 1995), female subjects and their partners were moved from their home cage in the colony room to the experimental room 48 hours prior to the PET scan to reduce the possible effect of novel housing on behaviour, neural and endocrine activity. The metabolism room/testing cage was 45.7 cm wide, 69.8 cm tall, and 61.0 cm deep. Animals were fasted 6–12 h prior to the scan, with water available throughout the pre-scan period.

At 0830 hours, the test animals were caught and removed from their cage. They received a bolus injection of [^{18}F]-fluorodeoxyglucose (FDG, PETNET Solutions, Sacramento, CA) (up to 2 mCi/kg IV, administered in a volume of <2 ml) into the saphenous vein. Each subject was returned to their cage for 30 min of conscious uptake either with their partner; with a previously unencountered adult male titi monkey (here referred to as a stranger), age-matched to their pair-mate; or alone, depending on the condition.

After the FDG uptake period, subjects were anesthetized with ketamine (25 mg/kg IM) and administered medetomidine (0.05 mg/kg IM). Immediately following sedation, 1 ml blood was drawn from the femoral vein. Time (mean ± standard error, hereafter abbreviated as SEM) from initial disturbance to blood collection averaged 3.30 ± 0.38 min across the three conditions. Atipamazole was used to reverse medetomidine, and anesthesia was maintained with isoflurane (1–2%) while the female was positioned on the scanner and throughout the scan. PET imaging was performed on Pi-PET (Brain Biosciences, MA), a commercial high-resolution PET device for dedicated brain imaging in non-human primates. Animals were maintained in metabolism cages for a day after scanning (until radiation had decayed), and then returned to their home cages with their male partners.

2.3.2. PET and MRI Co-registration and quantification of FDG Uptake.—

Boundaries for the ROI structures, as well as for the whole brain, were drawn on each subject's MRI image using Siemen's Inveon Research Workplace software (IRW, Siemens Healthcare, USA) by methods previously described in Arias-del Razo et al. (2020). Static PET images were reconstructed with a 3DRP reconstruction protocol. MRI images were co-registered with PET scan images using the automatic rigid registration algorithm in IRW and checked visually for acceptable registration accuracy. Mean FDG activity was determined by applying the ROI boundaries that were defined on the MRI images to the PET images in IRW. Finding no hemispheric differences in glucose uptake, ROI values were averaged across the hemispheres and were normalized by dividing by whole brain uptake.

2.3.3. Behavioural Observations—

Behavioural observations were scored for the first 25 minutes of FDG conscious uptake using continuous focal sampling (Altmann, 1974) of behaviours described in Supplementary Table 1. Depending on the experimental condition, after radiotracer injection, females returned to the metabolism cage together with their partner (partner condition), to an empty cage (alone condition) or with an unknown male (stranger condition). In order to eliminate the possibility of physical injury, the subject and stimulus animals were separated by a plexiglass divider during this uptake period.

Female location was scored as the time in seconds that the test female spent 15 cm or closer to the middle divider ("proximity"), the time in seconds that both animals were within 15 cm to the divider ("mutual proximity"), and the time in seconds that the female was not within 15 cm of the divider ("no proximity"). The frequency that the test female entered into proximity, mutual proximity, and no proximity was also scored. Subject proximity and mutual proximity were scored as mutually exclusive. Female affiliative and arousal behaviours such as lip-smack and back-arch, were scored as frequencies, as were chest-rub, touch of the plexiglass divider, and aggression (Table S1). The duration of touch of the plexiglass divider was also scored.

2.3.4. Cortisol assays—Blood samples were collected immediately following the period of conscious FDG uptake, as described above. A baseline sample was also collected approximately one week prior to the 1st PET scan, in order to document that the "alone" condition of the scan produced a stressful condition relative to home cage conditions. During baseline blood sampling, each female was boxed and handled for sampling directly from her home cage, and these samples were collected within 2.51 ± 0.22 min (mean ± SEM) after cage disturbance. Samples were collected, placed on ice, and centrifuged, and plasma was removed and stored at −80°C. Cortisol was assayed by the Clinical Endocrinology Laboratory at the UC-Davis Veterinary School, with an enzyme immunoassay (antibody R4866, produced by UC-Davis Clinical Endocrinology Lab), which has been validated for female titi monkeys both chemically, and biologically by ACTH and dexamethasone challenge (Witczak et al., 2021). Cortisol was diluted at 1:1000; samples were assayed in duplicate. Inter-assay c.v.s were 1.27% and intra-assay c.v.s were 4.00%.

2.3.5. Statistical analyses—Behavioural and brain models contained three conditions (alone, partner, and stranger) while the cortisol model contained four conditions (baseline, alone, partner, and stranger). We used mixed effects models to examine changes in these variables across the conditions. Given that these conditions were counter-balanced (except for baseline; see dates in Supplementary Table 2), in addition to the main contrasts, our models examined order effects, age and years of pairing. For cortisol we then made four comparisons between the conditions: a) baseline vs all conditions, b) alone vs paired (partner or stranger), c) partner vs stranger, and d) alone and stranger. Brain and behavioural models did not contain comparison a.

We evaluated hypotheses using dummy variables representing the specific comparisons (see Supplementary Table 3 for more information on models used). ß coefficients were unstandardized. Effect sizes were calculated as Cohen's d for mean comparisons, and as percentages of reduced unexplained variance for regression models, including interactions. We evaluated the statistical significance of results using this modeling approach, while simultaneously evaluating biological significance given the context, effect sizes, and description of the results, in order to best interpret our outcomes.

3. Results

3.1. Behaviour

a) Females entered the mutual proximity zone more frequently with their partner than with the stranger male

Mutual proximity: Mutual proximity was the amount of time that females spent at the divider while the male subject was directly on the opposite side. The frequency with which females entered into mutual proximity with their partners was significantly higher in the partner condition compared to the stranger condition, with a small/medium effect size (ß= 3.74, p=0.03; Cohen's d=0.41; Figure 1). Although the duration that females spent in mutual proximity did not differ significantly between their partner and the stranger male, the effect size was moderate (ß=63.68, p=0.19; Cohen's d=0.60), not surprisingly, as the mean (±

SEM) for duration of mutual proximity was 334.5 ± 103.47 for the partner and 194.12 ± 54.0 for the stranger.

In other words, females approached their partner more often than the stranger when the respective male was next to the divider. Once at the divider, they did not spend significantly more time in mutual proximity with the partner than with the stranger, although the relatively large effect size suggests that this comparison may be under-powered, that there may be a lot of variation in individual behaviour, or both (see below).

Proximity: "Proximity" was scored exclusively of mutual proximity and was therefore the time that females spent at the divider while there was no stimulus animal near the other side. This behaviour might be interpreted as the amount of "unrequited" interest on the part of the female subject in interacting with the male stimulus animal, or as an attempt to monitor the male. There was no significant difference in the duration (sec) that females spent in proximity to the divider in the three conditions. Females did not spend significantly more time in proximity to the divider when alone compared to when in the paired conditions (ß=−49.86, p= 0.43; Cohen's d=0.11); when comparing partner and stranger condition (ß=4.80, p=0.94; Cohen's d=0.42); and finally, when comparing alone with the stranger condition (ß=19.71, p=0.78; Cohen's d=0.09); Figure 2). The differences between groups were negligible, except perhaps when comparing partner and stranger condition; females in the partner condition spent 495.37 ± 124.46 sec in proximity to the divider compared to 364.2 ± 93.09 sec when they were in the stranger condition.

As we found for duration, the frequency with which females entered into proximity was not statistically different in the three conditions (alone vs pair: ß=3.05, p=0.29; partner vs stranger: ß=2.78, p=0.29; alone vs stranger: ß= −0.85, p=0.69; Cohen's d=0.16). A moderate effect size was found when comparing alone and paired conditions (Cohen's d=0.41), and partner and stranger conditions (Cohen's d=0.46).

On a descriptive level we found large individual differences in the time spent in proximity to the plexiglass depending on the individual female and the condition (Figure 2). For instance, two test females spent remarkably more time in proximity to the divider when in the partner condition compared to the stranger condition. One female spent 141.88% more time in proximity to her partner compared to the stranger animal, while the other female spent 1,978% more time in proximity when in the partner condition. A third female spent 249% more time in proximity to the divider in the stranger condition compared the partner condition.

Years of pairing had an effect on the time spent in proximity in the model for partner vs. stranger condition (ß=64.19, p=0.03, 2.5% decrease in unexplained variance), and on the frequency to enter the proximity zone when comparing the alone condition with the stranger condition (ß=2.65, p=0.04, 0.5% decrease in unexplained variance). In other words, for each year that the female had been with her current pair-mate, she spent 2.5% more time in proximity to the divider during the partner condition than the stranger condition. Likewise, for each year the female had been with her current pair-mate, she approached the divider

0.5% more frequently in the alone condition than in the stranger condition. Age did not influence these outcomes (Supplementary Table 4).

Total proximity: Because "proximity" was mutually exclusive from "mutual proximity", their sum was equal to the **total** time spent near the divider by the subject. The duration of "total proximity" was higher in the partner condition at a mean (±SEM) of 829.88 ± 139.70 compared to 558.38 ± 125.26 in the stranger condition, and 393.25 ± 124.96 in the alone condition. Females entered into "total proximity" an average of 42.38 ± 5.73 times in the partner condition, 33.75 ± 6.98 times in the stranger condition and 17.25 ± 3.81 times in the alone condition. When comparing the partner and stranger condition, there was a moderate effect size but marginal p-values in the time spent in total proximity (Cohen's d=0.72, ß=2.19, p=0.09), and in the frequency of entering the proximity zone (Cohen's d=0.48, ß=6.73, p=0.06). Thus, overall these results are suggestive that females preferred to be close to the divider in the partner condition than in the stranger or alone conditions, whether or not their mate was also sitting next to the divider.

No proximity: Finally, females did not differ in the time spent in "no proximity", or the frequency of entering, the "no proximity" zone depending on condition (Supplementary Table 4).

b) Females did not show more affiliative behaviour towards their partner

—Females did not differ significantly in affiliative or arousal behaviours when in the partner condition than in the stranger condition, although p-values are marginal: including lip-smacking (ß=−2.73, p=0.10), back-arching/tail-lashing (ß=−2.62, p=0.09), duration of actively touching the divider (ß=29.59, p=0.15), and frequency of actively touching the divider (ß=7.75, p=0.08) (Figure 3). Effect sizes were small to moderate. Females lip-smacked more to the stranger animal (Cohen's d=0.40) at a mean (±SEM) of 6.38 ± 3.66 times, compared to 3 ± 2.35 times in the partner condition and 0.88 ± 5.94 times in the alone condition. Frequency of back-arching/tail-lashing was higher in the stranger condition (Cohen's d=0.74; 5.5 ± 3.15 times) compared to 0.75 ± 0.62 times in the partner condition. Females did not show any back-arching/tail-lashing during the alone condition.

In contrast, females touched the divider more frequently (Cohen's d=0.46, 21.5 ± 12.32 times) in the partner condition compared to 9.13 ± 5.28 times in the stranger condition. Females touched the divider for longer duration (Cohen's d=0.40) in the partner condition, at a mean (±SEM) of 89.89 ± 52.88 seconds compared to the stranger condition with a mean of 33 ± 20.15 seconds. No females showed any aggression or chest rubbing.

In the model which compared alone vs. social conditions, age significantly predicted back-arching/tail-lashing, which went down with age (Supplementary Table 4). The same contrast (alone vs. social) was significant for time spent touching the divider as were the years spent paired (Supplementary Table 4). Females in the social conditions spent more time touching the divider at a mean (±SEM) of 61.43 ± 36.51 sec compared to the alone condition at 47.63 ± 29.44 sec.

3.2. While the presence of either male reduces plasma cortisol compared to being alone, spending more time near the stranger results in higher plasma cortisol

Cortisol levels showed an effect of order (ß= 663.64, p= <0.0001). However, this order effect could be explained by the lower values of the baseline condition, which were always collected first (Figure 4). The effect was non-linear (0, .80, .68, 1), with a rapid increase from the first to the second condition (80% of the effect), a slight decrease to the third condition (about 12%), and a final increase to the fourth condition (32%).

There was a significant effect of order in all models except that of partner vs. stranger (Supplementary Table 5). We also found order effects in the interaction between stranger condition and proximity (ß= −4.68, p=0.02, 9.9% decrease in unexplained variance) and in the interaction between stranger condition and mutual proximity (ß= −8.45, *p*=0.049, 5.8% decrease in unexplained variance).

We found a significant effect of social condition on cortisol levels (Figure 4). Baseline condition did not significantly differ when compared to all three other experimental conditions (alone, partner, and stranger; ß= 3.51, p=0.98), most likely because of the strong order effect (above). There was a significant effect when comparing the alone condition with the paired condition (partner and stranger; ß= −130.60, *p*= 0.001; Cohen's d=1.07), with a large effect size, cortisol being higher in the alone condition than in either of the social conditions. There was also a significant difference and a large effect size when the alone condition was compared to the stranger condition (ß= 88.71, *p*= 0.04; Cohen's d=1.07). Surprisingly, cortisol did not differ in the partner condition compared to the stranger condition (ß= −7.50, *p*= 0.78; Cohen's d=0.04). We did not find any effects of age or years of pairing on cortisol models (Supplementary Table 5) except when comparing baseline with the other three conditions, in which years of pairing had a significant effect, with cortisol going down with additional years of pairing (ß=−32.10, *p*=0.05, 4.0% decrease in unexplained variance).

Females in the stranger condition showed higher cortisol the longer that they spent in proximity to the divider on the stranger side (ß=3.98, p=0.01; 8.1% decrease in unexplained variance). The interaction between the partner condition and time spent in proximity to the partner side did not predict plasma cortisol changes (ß=−1.59, p=0.11; 0.6% decrease in unexplained variance).

The interaction between stranger condition and time spent in mutual proximity (both the female and the stranger at the plexiglass divider) also predicted changes in plasma cortisol in the females, being higher with more time spent at the divider (ß=6.48, *p*=0.046; 8.2% decrease in unexplained variance; Figure 5). We did not find a relationship between time in mutual proximity and cortisol levels for females in the partner condition (ß=−1.70, p=0.21; 0.4% decrease in unexplained variance).

3.3. The stranger male condition results in increased glucose uptake in the female's amygdala and hippocampus

We found no effects of experimental condition on brain metabolic activity in the LS, NAcc, putamen, caudate, ACC, PVN, or SON when comparing alone and pair conditions; alone

and stranger condition; and finally, partner and stranger condition (Supplementary table 6; Figure 6). Brain metabolic activity in the amygdala did not differ between alone condition and the social conditions (ß=0.01, *p*=0.25; Cohen's d=0.32). However, amygdala activity was significantly higher for females in the stranger condition both when compared to alone condition (ß=−0.02, *p*=0.02; Cohen's d=0.69) and when comparing the stranger to the partner condition (ß=−0.02, *p*=0.02; Cohen's d=0.60), with a moderate effect size.

A similar effect was found in the hippocampus. We did not find an effect on hippocampal glucose uptake when comparing alone with both social conditions (ß=0.01, *p*=0.40; Cohen's d=0.15). A comparison between the alone condition and the stranger condition was marginally significant (ß=−0.01, *p*=0.06; Cohen's d=0.53). However, hippocampal uptake was significantly (by approximately 4.5%) higher for females when in the stranger condition compared to the partner condition (ß=−0.02, *p*=0.01; Cohen's d=0.54).

There were no order or age effects on brain glucose uptake (Supplementary Table 6). Years of pairing had an effect on uptake in the putamen when comparing alone with stranger condition (ß=−0.01; *p*=0.04, 5.0% decrease in unexplained variance). More years of pairing were associated with lower uptake in the putamen.

Time that the subject spent in proximity to the partner's side predicted higher uptake in the LS (proximity: ß=0.00017, p=0.004; 7.5% decrease in unexplained variance), hippocampus (proximity: ß=0.0002, *p*=0.02; 5.6% decrease in unexplained variance) and NAcc (proximity: ß=0.00018, p=0.047; 4.6% decrease in unexplained variance). However, mutual proximity in the partner condition did not predict higher uptake in the LS (ß=−0.00009, p=0.17), hippocampus (ß=−0.0002, p=0.15) or NAcc (ß=−7.61E-6, p=0.95; 1.3%, 1.7%, and 0.3% decrease in unexplained variance, respectively). The relationships between time spent in proximity and mutual proximity to the stranger animal did not statistically predict higher uptake in the amygdala, although the results for proximity were marginal (proximity: ß=0.00012, p=0.055; mutual proximity: ß=−0.00002, p=0.77; 1.9% and 0.9% decrease in unexplained variance, respectively).

4. Discussion

This study was the first investigation to concurrently combine neural, hormonal, and behavioural responses of pair-bonded female titi monkeys to their partner vs. a stranger, vs. time spent alone. Some of the results were surprising. For instance, females did not engage in higher levels of affiliative and arousal behaviour with their partner than with a stranger male; and stranger males appeared to be able to buffer the females' cortisol response to stress. However, females approached their partners more frequently than they approached a stranger male. They also touched the divider between themselves and their partner more often and for more time, presumably trying to gain physical access to their partners. As we would have predicted, more time physically near the stranger predicted higher cortisol levels in females, and this was not true for the time spent near the partner. Finally, we found that when interacting with stranger males, female titi monkeys showed increased glucose uptake in the amygdala and hippocampus compared to interacting with partner males.

Examining the females' choice of location was complicated, although in general females showed preference for maintaining proximity to their partner over a stranger male. "Mutual" proximity would also be affected by their partners' behaviour, and here we did not consider the behaviour as a dyadic process. However, since frequency of entering the "no proximity" zone did not differ between groups, we do not attribute these differences to baseline locomotion.

Females tended to display more lip smacking and back arching towards the stranger male compared to their partner, suggesting that they were both aroused and acting in a friendly manner. However, they displayed more divider touching when in the partner condition, presumably trying to touch him. Due to the plexiglass dividers, we were unable to observe tail-twining, possibly the most important affiliative behaviour in reflecting the "intimacy" of the attachment (Arias-del Razo et al., 2022a). This behaviour would have given us additional insight into the differences in the reaction between the females and their partner vs. the stranger animal. We did observe large individual differences in behaviour between females in almost all behavioural measures. For instance, two females, both paired for less than two years, showed more affiliative/arousal behaviours (lip smacking and arching) towards the stranger male. The oldest and longest-paired females showed fewer affiliative and arousal behaviours towards both their partner and the stranger animal compared to the younger ones/paired for less than two years. Years of pairing did statistically influence several of our outcomes and should be included in future studies.

As we expected, females in the partner condition showed lower plasma cortisol compared to females which were alone for 30 minutes. Interestingly, we found that the stranger animal also buffered females against the rise in cortisol. Females may have seen the stranger male as a possible sexual partner instead of as a danger. In studies on both wild-living and captive titi monkeys, mated individuals occasionally engage in sexual activity with stranger individuals (Anzenberger, 1988; Fernandez-Duque, Mason & Mendoza, 1997). Moreover, the stranger males in our study were mostly inactive, and they did not show aggressive behaviour towards the experimental female. There was also no aggression from the female towards the stranger male. While both mate guarding and intersexual aggression are common in some other socially monogamous species (Carter et al., 1995), overt aggression is rarer in titi monkeys (Mason, 1966), especially in females. Ultimately, the presence of a benignly behaving stranger male appears to have been less stressful for females than being alone in a novel environment.

Despite what appeared to be a stress buffering effect of the stranger male, the females' cortisol levels were higher the longer that they spent in mutual proximity with the stranger. An alternative possibility is that stranger males were less effective buffers against stress and that given more time, the females' cortisol levels would have eventually equalled or surpassed the levels measured in the alone condition. In male titi monkeys, the longer that subject males looked at their female pair mates interacting with a stranger male, the higher their plasma cortisol concentrations (Maninger et al., 2017b). Therefore, it is also possible that more subtle aspects of gaze and location might have affected our plasma cortisol outcomes.

In contrast to our predictions, we did not find significant differences in glucose uptake between partner, alone, and stranger conditions in dopaminergic areas. We did find that FDG uptake in the amygdala and the hippocampus was significantly higher during the stranger condition. In addition to the emotional processing of fear, anxiety, and arousal, the amygdala as well as the hippocampus are involved in cognitive processes, such as memory or attention, that also have an emotional component (Gallagher & Chiba, 1996; Phelps, 2004). These females were encountering a new male and possibly creating a new emotional memory for future social recognition.

Time spent in proximity to the partner's side (but not mutual proximity with the partner) predicted higher FDG uptake in the LS and NAcc, as well as in the hippocampus. These areas play an important role in the formation of a pair bond in male titi monkeys (Bales et al., 2017; Maninger et al., 2017a). These brain areas contribute directly to the "socio-spatial memory neural circuit" (Ophir, 2017). This brain circuit shapes mating and bonding behaviours and is involved in learning, social recognition, and spatial memory (Ophir, 2017; Montagrin et al., 2018). The interaction between proximity and FDG uptake in these areas might be caused by their motivation to look for social proximity and cope with the separation stress (Bales et al., 2017) caused by the divider.

Comparisons with results from male titi monkeys should be made with caution based on differences between experimental designs; however, it is notable that in studies where male titi monkeys have encountered stranger females, different patterns of neural glucose uptake emerged (Bales et al., 2017). Male titi monkeys encountering a stranger female primarily showed decreases in glucose uptake in the ventral pallidum; while when reuniting with their partner, they showed decreases in the medial amygdala and central amygdala, as well as the SON and PVN (Hinde et al., 2016). However, these social encounters followed a two-week separation from the partner, unlike the current study which was not prefaced by a separation.

In future studies, a change in the experimental paradigm could give valuable insights into how experimental and external factors can affect neural, physiological, and behavioural results. Motor and visual activity as well as the partner and stranger males' behaviours should be scored with the purpose to analyse changes in females' reactivity towards the new social condition. Male behaviour could have contributed to the difference in plasma cortisol as well as in brain FDG uptake and behaviour. As we can see in our results, female titi monkeys behave in a heterogeneous manner, which could have contributed to the variation in our other outcome measures. Parenthood status should be considered in future analyses as well, as other studies have reported that neural glucose uptake and behaviour differed depending on this factor (Hinde et al., 2016). Finally, it would be interesting to see if the number of mates that the female had in the past (and therefore her level of comfort with new males) might affect their interaction with a stranger animal.

5. Conclusions

To conclude, both the presence of the partner as well as that of the stranger, acted as social buffers in a novel stressful situation. Females in the alone condition showed substantially higher plasma cortisol levels compared to the partner and stranger conditions. We found

significant differences in FDG uptake in the amygdala and hippocampus, which were higher in the stranger condition. While females entered mutual proximity more frequently and tended to spend more time closer to their partner, they showed more arousal/affiliative behaviours to the stranger animal. To completely understand these results, further studies on the maintenance of female social bonds will be needed. This comes with the need to include factors such as locomotion, gaze patterns, home cage social behaviour, length of pairing, age, and their parenthood status using a larger sample. We should also consider that titi monkeys are individually variable in their behaviour, and new factors should be considered to better understand these individual differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data are available in Supplemental Table 2.

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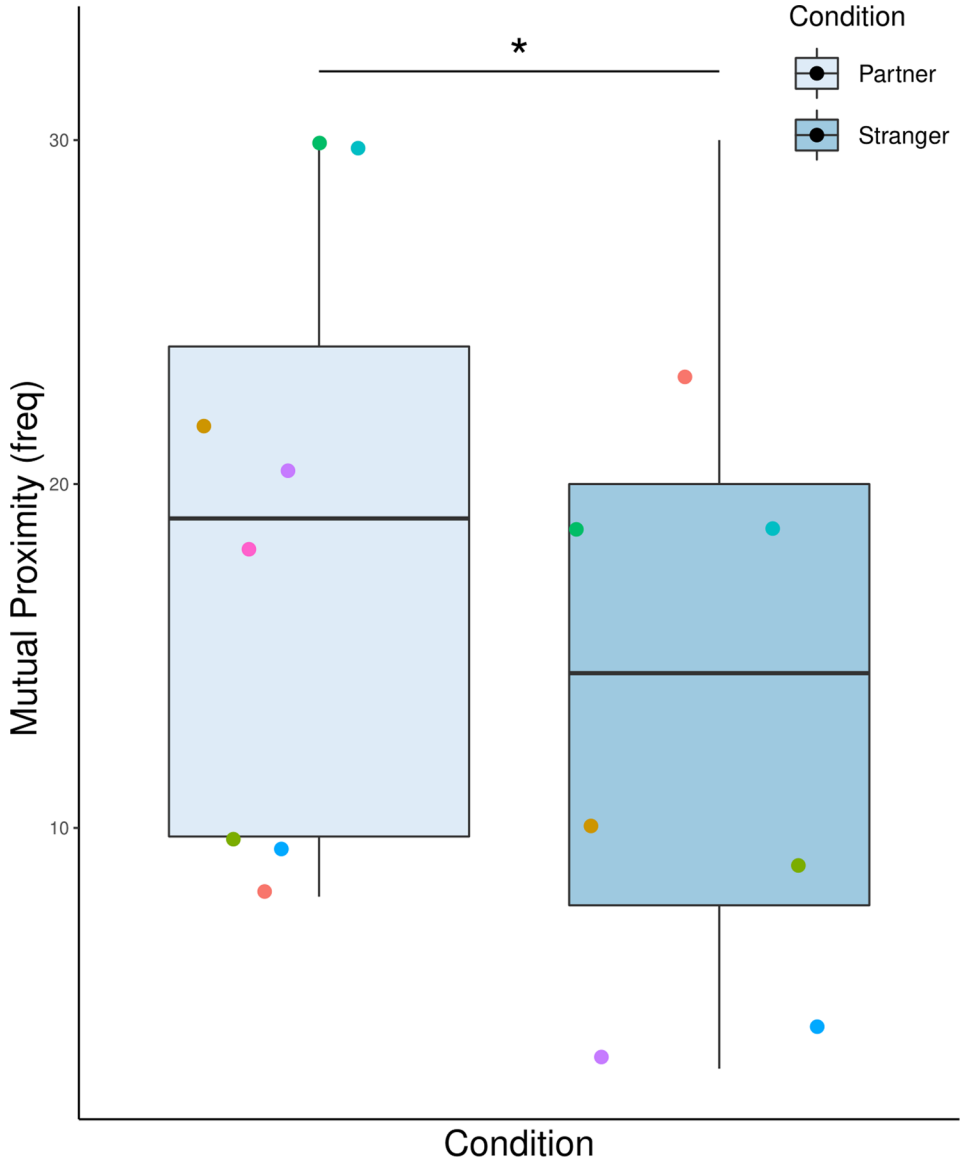

Figure 1.

Frequency of entrances into mutual proximity (frequency). Comparisons were made between partner and stranger condition (β=3.74, *p*=0.03). Each color dot represents an individual. Box plots show medians and interquartile ranges. The *p* value was set as 0.05*, 0.01** and 0.001***.

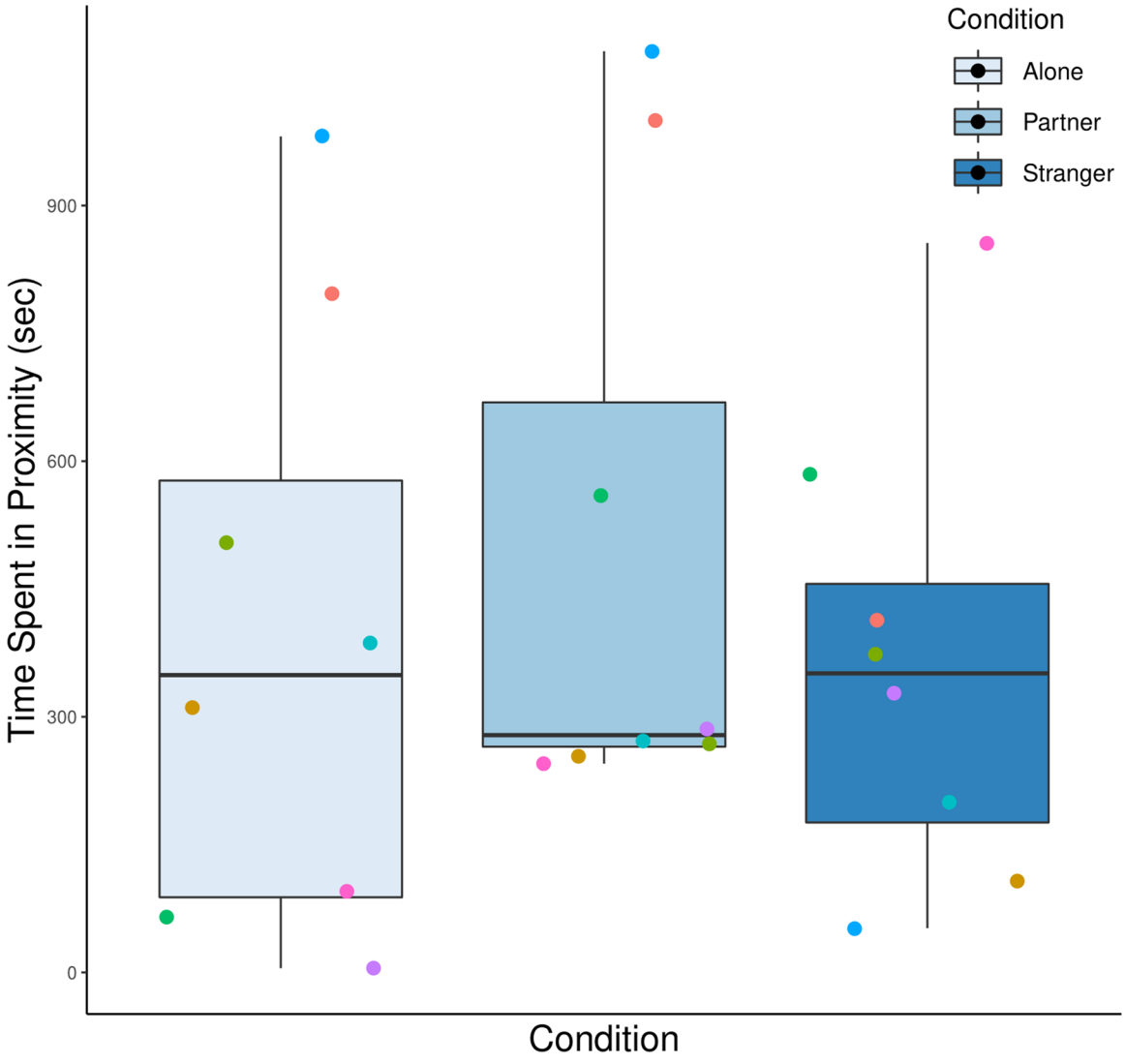

Figure 2.

Time spent in proximity to the plexiglass depending on condition. Comparisons were made between partner and stranger condition (β=4.80, p=0.94). Each color dot represents an individual. Box plots show medians and interquartile ranges. The p value was set as 0.05*, 0.01** and 0.001***.

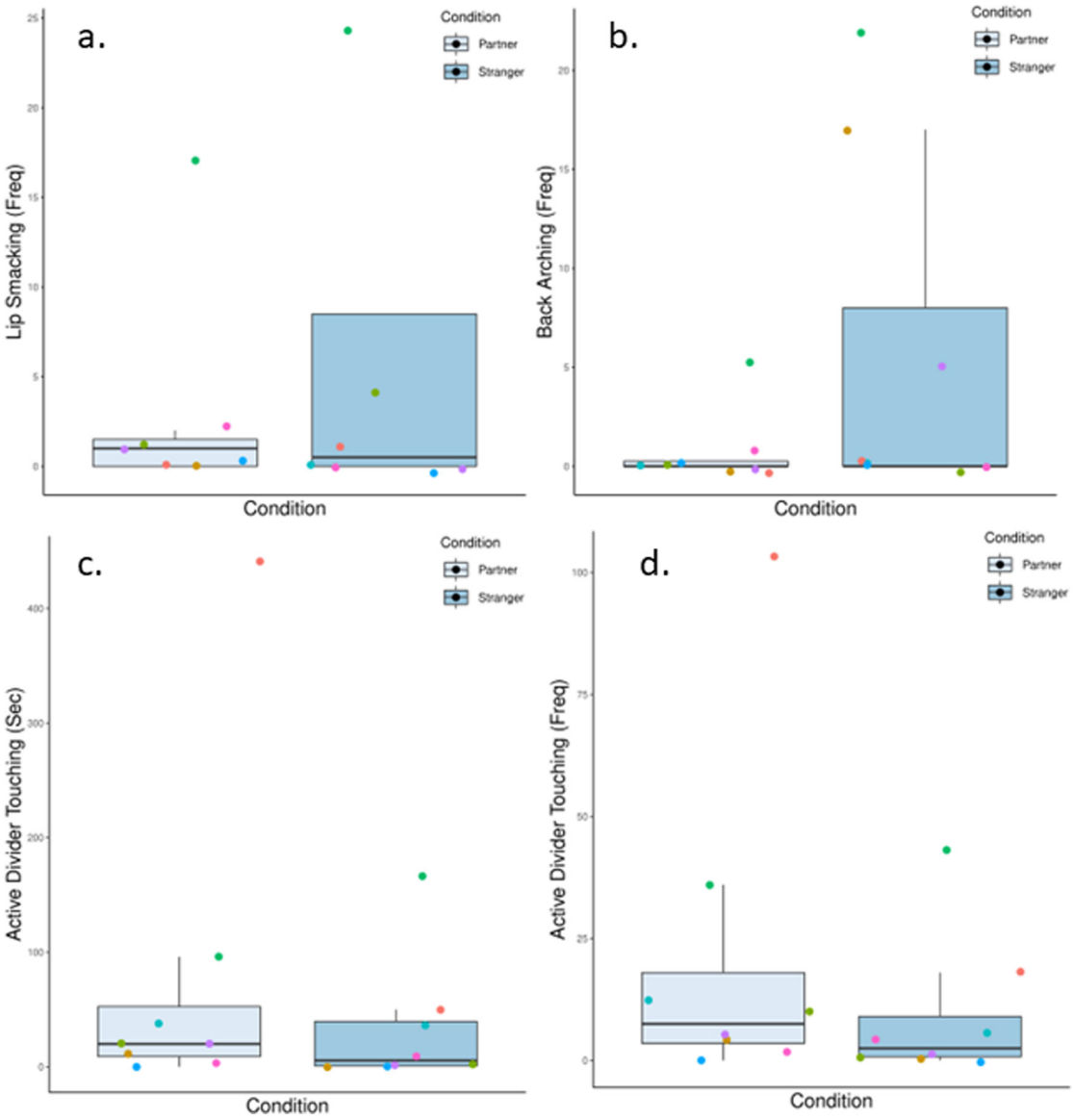

Figure 3.

Changes in affiliative and arousal behaviour depending on condition. Comparisons were made for a) lip smacking, b) back-arching, c) active divider touching (duration), and d) active divider touching (frequency). Each color represents an individual. One female in the partner condition was excluded from the analyses in lip smacking affiliative/arousal behaviour due to low video quality. Each color dot represents an individual. Box plots show medians and interquartile ranges. The *p* values were set as 0.05*, 0.01* and 0.001***.

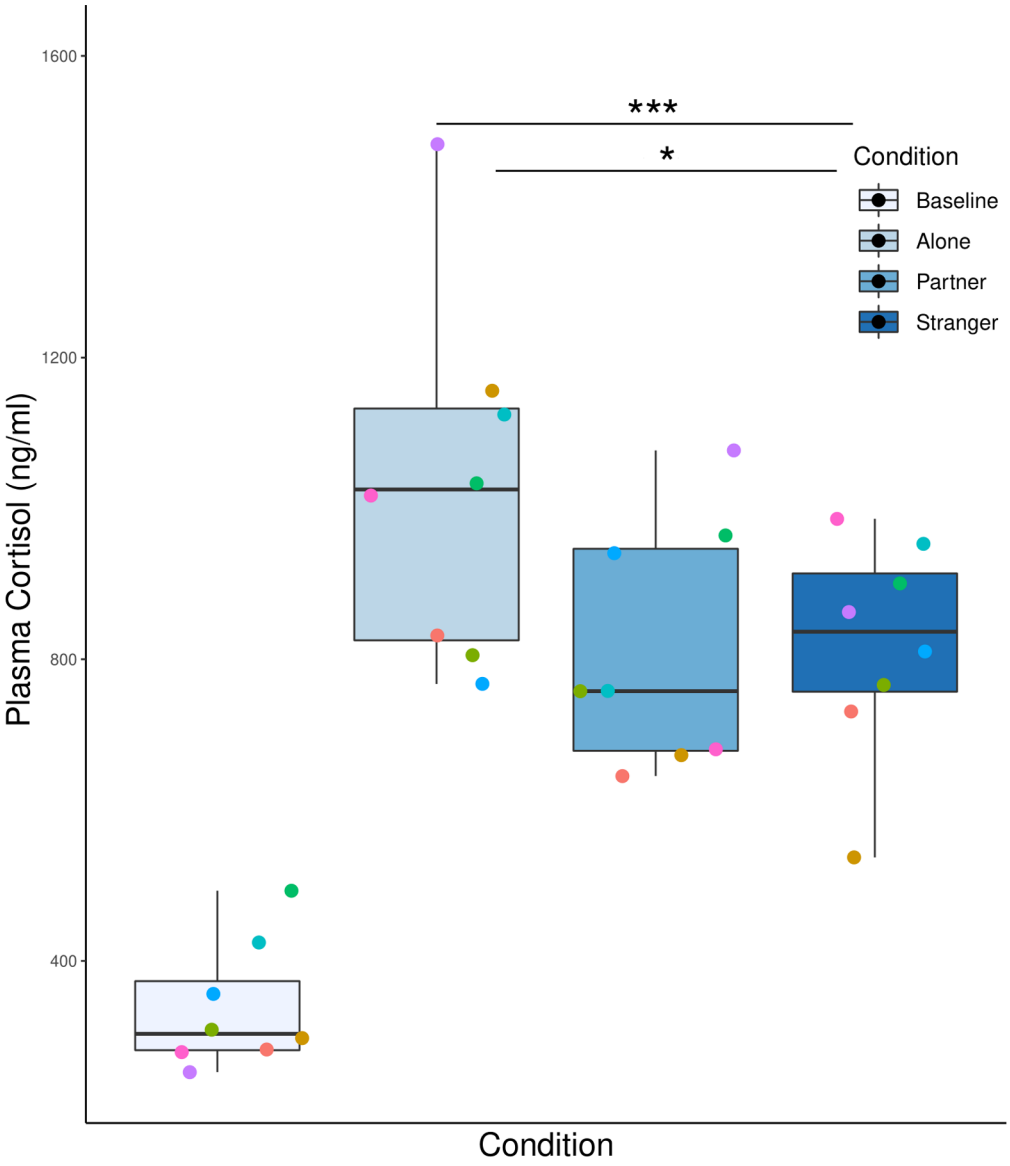

Figure 4.

Baseline cortisol values and cortisol values in response to social experimental conditions. Comparisons were made between females in the partner condition (N=8) and females alone (N=8) or with a stranger animal (N=8). Each color represents an individual. Box plots show medians and interquartile ranges. The *p* value was set to 0.05*, 0.01** and 0.001***.

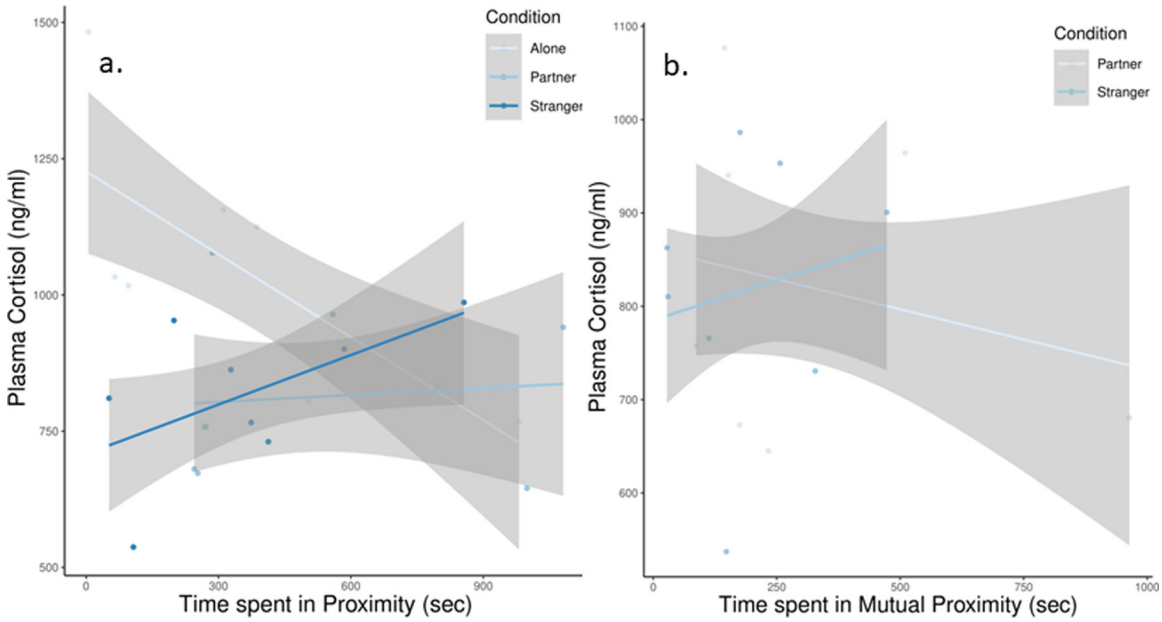

Figure 5.

Change in plasma cortisol levels in response to social experiment conditions and **a)** time spent in proximity to the plexiglass, **b)** time spent in mutual proximity to the male animal. Analyses were made between females in the partner condition (N=8) and females alone (N=8) or with a stranger animal (N=8). The *p* value was set to 0.05*, 0.01** and 0.001***.

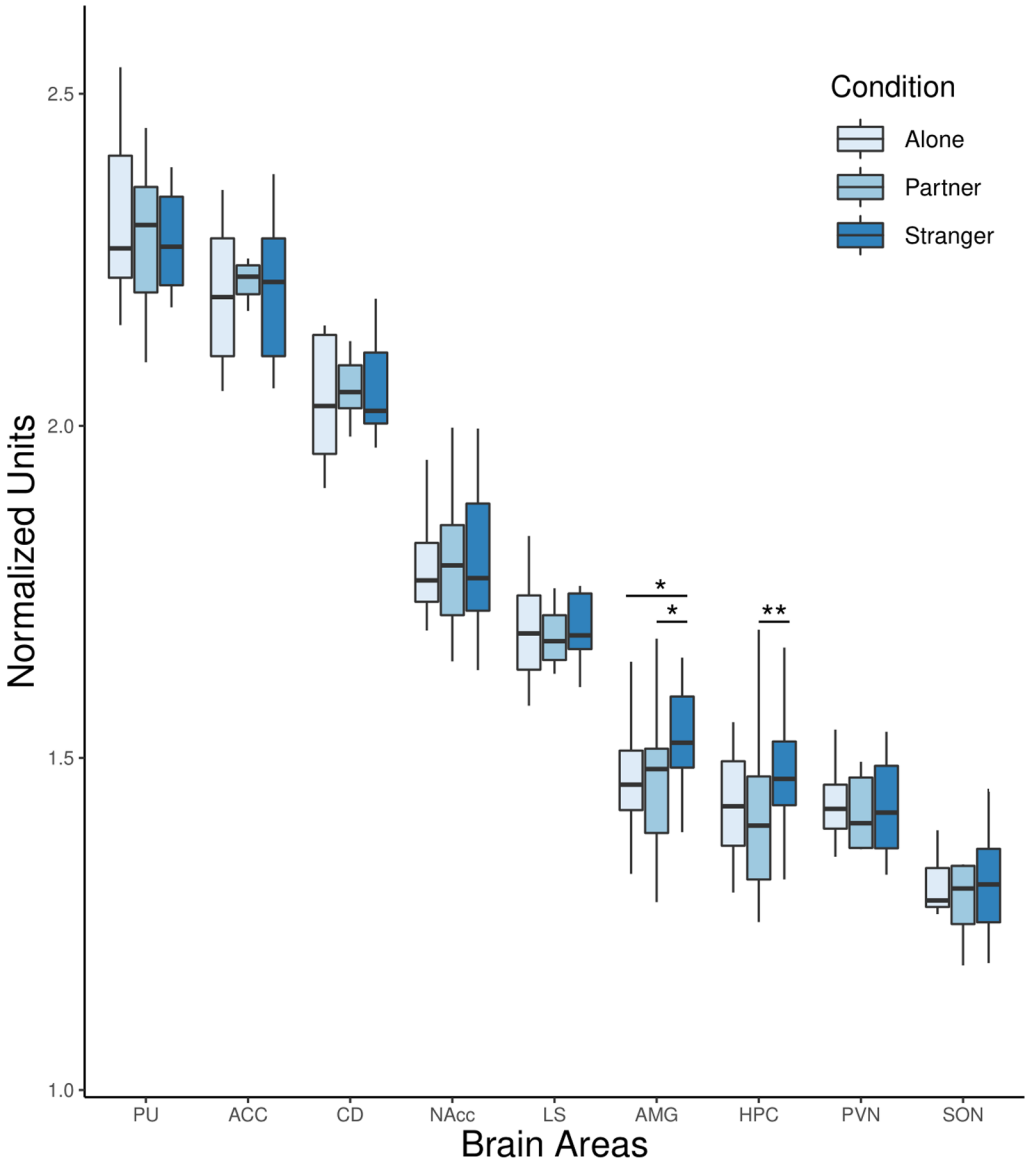

Figure 6.

Changes in brain FDG uptake depending on social condition. Box plots show medians and interquartile ranges. The *p* value was set as 0.05*, 0.01** and 0.001***. [Brain areas: PU = putamen, ACC = anterior cingulate cortex, CD = caudate, NAcc = Nucleus accumbens, LS = lateral septum, AMG = amygdala, HPC = hippocampus, PVN = Paraventricular nucleus, SON = supraoptic nucleus].

Table 1.

Subject Characteristics

Subject ID	Subject age	Number of years paired	Age difference with the male partner (years)	Female's first pairing?	Offspring in cage?
Subject 1	19.08	2.00	3.67	N	N
Subject 2	15.75	6.17	0.42	N	N
Subject 3	11.58	2.42	5.00	N	N
Subject 4	11.08	6.75	2.67	N	Y
Subject 5	10.17	0.92	0.08	N	N
Subject 6	7.92	0.42	8.83	N	N
Subject 7	3.25	1.42	0.42	Y	N
Subject 8	3.08	0.75	3.67	Y	N