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Pair bond Formation Leads to a Sustained Increase in Global Cerebral Glucose Metabolism in Monogamous Male Titi Monkeys (*Callicebus cupreus)*

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Abstract

Social bonds, especially attachment relationships, are crucial to our health and happiness. However, what we know about the neural substrates of these bonds is almost exclusively limited to rodent models and correlational experiments in humans. Here, we used socially monogamous non-human primates, titi monkeys (*Callicebus cupreus*) to experimentally examine changes in regional and global cerebral glucose metabolism during the formation and maintenance of pair bonds. Baseline positron emission tomography (PET) scans were taken of thirteen unpaired male titi monkeys. Seven males were then experimentally paired with females, scanned and compared, after one week, to six age-matched control males. Five of the six control males were then also paired and scanned after one week. Scans were repeated on all males after four months of pairing. PET scans were coregistered with structural magnetic resonance imaging (MRI), and region of interest (ROI) analysis was carried out. A primary finding was that paired males showed a significant increase in FDG uptake in whole brain following one week of pairing, which is maintained out to four months. Dopaminergic, “motivational” areas and those involved in social behavior showed the greatest change in glucose uptake. In contrast, control areas changed only marginally more than GCGM. These findings confirm the large effects of social bonds on global cerebral glucose

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metabolism. They also suggest that more studies should examine how social manipulations affect whole brain FDG uptake, as opposed to assuming that it does not change across condition.

Keywords

pair bond; imaging; PET; global cerebral glucose metabolism

Introduction

Our ability to form and maintain social relationships is crucial to our health, psychological well-being, and reproductive fitness (Uchino et al., 1996, Uchino, 2006). These relationships start with our initial attachment to our caregivers, and expand past our family circle with friendships as juveniles and adolescents. As adults, we may maintain multiple strong, selective social bonds including romantic attachments, friendships, and bonds with both our parents and our children.

The neural basis of relationships has primarily been studied in the context of parent-offspring or adult romantic relationships (Carter, 1998, Bartels and Zeki, 2000, Rilling et al., 2001, Bartels and Zeki, 2004, Acevedo et al., 2012). Adult romantic relationships, or pair bonds, can be conceptualized in the same way as offspring to parent attachments (Hazan and Shaver, 1987, Mason and Mendoza, 1998). As such, they involve a preference for the attachment partner, distress upon separation, and the ability of the attachment partner to buffer the subject against stress (Bowlby, 1969, Ainsworth et al., 1978). In non-human animals, these characteristics of a pair bond have only been demonstrated in a limited number of species, all of which display social monogamy, a social system in which a (usually male-female) pair shares a territory and reproduces together, generally with the aggressive exclusion of other non-related adults of their species (Kleiman, 1977, Fuentes, 1999, Diaz-Munoz and Bales, 2016). Prairie voles (*Microtus ochrogaster*) and titi monkeys (*Callicebus cupreus*) are two of these species.

Prairie voles have contributed most of our knowledge on the neurobiology of pair bonding (Gobrogge and Wang, 2015). In brief, pair bond formation is associated with the release of the peptide hormones oxytocin (OT) and vasopressin (AVP) from the hypothalamus (Winslow et al., 1993, Cho et al., 1999, Liu and Wang, 2003, Ross et al., 2009). OT and AVP are crucial for pair-bond formation in both sexes (Winslow et al., 1993, Williams et al., 1994, Cho et al., 1999, Lim et al., 2004, Lim and Young, 2004, Ross et al., 2009, Johnson et al., 2015), presumably because of their association with the formation of social memories (Engelmann et al., 1996, Bielsky et al., 2004). In prairie voles (but not polygamous voles such as meadow voles), the receptors for OT and AVP (V1a) are co-localized with dopamine D2 receptors in areas of the reward system such as the nucleus accumbens and ventral pallidum (Insel and Shapiro, 1992, Insel et al., 1994). Co-activation of OT or AVP (i.e. for social memory) and D2 (i.e. for Motivational) results in pair bond formation (Aragona et al., 2003, Aragona and Wang, 2009). Following this formation, dopamine D1 receptors are up-regulated along with aggression towards outsiders, a.k.a. mate-guarding (Aragona et al., 2006). Imaging studies of humans, in which subjects are thinking about the object of their

romantic love, concur in implicating the reward system (Bartels and Zeki, 2000, Xu et al., 2011, Acevedo et al., 2012), and other hypothalamic areas (Acevedo et al., 2012).

Prairie voles are the only system in which the neurobiology of pair bonding has been studied in detail (Carter et al., 1995, Gobrogge and Wang, 2015). While they make an excellent model due to their clearly monogamous social system, some aspects of these findings have not held up in a wider taxonomic context. For instance, repeats within the vole promoter region of the gene for the AVP V1a receptor were found to predict the degree of pair bonding (Hammock and Young, 2005, Hammock et al., 2005). However, this pattern was not found across a wider sample of mammalian species (Fink et al., 2006). For this reason, our knowledge of the neurobiology of pair bonds should not be limited solely to one rodent model (Phillips et al., 2014). In this study, we utilized the titi monkey, a monogamous primate.

Titi monkeys, like prairie voles, live in nuclear family groups in nature. They duet, maintain small territories which they defend against intrusion, and the male is the main carrier of the infants (Mason, 1966, 1968, Mendoza and Mason, 1986b). Adult pairs display many characteristics of a pair bond, including preference for their partner (Carp et al., 2016), distress upon separation (Mendoza and Mason, 1986a), and the ability of the partner to buffer stress (Mendoza et al., 2000). In a previous study, we showed that males that had been in long-term pairs, vs. males that were not paired, showed many differences in regional cerebral glucose metabolism (rCGM) using positron emission tomography (PET) with [^{18}F]-fluorodeoxyglucose (FDG)(Bales et al., 2007). We found FDG uptake differences between paired and lone males in the regions of the nucleus accumbens, ventral pallidum, medial preoptic area, medial amygdala, and supraoptic nucleus of the hypothalamus; while not finding differences in the central amygdala or periaqueductal grey. In that study, like many other studies, rCGM data were normalized to global cerebral glucose metabolism (GCGM), and were in units of proportion of whole brain FDG uptake (i.e. rCGM was divided by GCGM). However, because GCGM (the denominator of the equation) was higher in long-term paired males, this normalization caused it to appear that rCGM was lower in paired males in many areas when compared to lone males.

This previous study (Bales et al., 2007) was also cross-sectional, with paired males older than lone males. Thus, age was confounded with pairing status. The goal of the current study was to examine the neurobiology of pair bonding longitudinally over formation in titi monkey males, compared with age-matched controls when possible. As in our previous study, we used PET scans with conscious uptake of FDG. We hypothesized that after males were paired we would see increased global cerebral glucose metabolism (higher whole brain FDG uptake), with higher than average FDG uptake in regions of interest identified as either “reward” areas or “social memory” areas. We also examined areas that produce OT and AVP, as well as the prefrontal cortex which has recently been implicated in several studies of more “universal” or “altruistic” love (Mathur et al., 2010).

Experimental Procedures

All experimental procedures were approved by the Animal Care and Use Committee of the University of California, Davis, and complied with National Institutes of Health ethical guidelines as set forth in the Guide for Lab Animal Care.

Subjects

Subjects were 13 captive-born and reproductively naïve adult male titi monkeys (*Callicebus cupreus*) housed at the California National Primate Research Center (CNPRC) in Davis, CA. Subjects were removed from their natal group and housed alone for greater than one month prior to the beginning of the study. Animals were fed twice daily (0830 and 1330 h) a diet consisting of New World monkey chow, rice cereal, banana, apples, raisins, and baby carrots and water was available *ad libitum*. Further details of husbandry and training are available elsewhere (Tardif et al., 2006), with caging identical to that described in (Carp et al., 2016).

Experimental Design and Pair Formation

All 13 males (mean age 4.18 years, range 2–5.6 years) underwent a baseline “unpaired” positron emission tomography (PET) scan and blood draw (one male was removed afterwards from the study due to health reasons). After the unpaired scan, six males (mean age 4.32 years) were paired with females and six males served as their singly-housed age-matched controls (see experimental design in Figure 1). Newly-paired subjects and age-matched controls underwent another PET scan seven days after pair formation (experimental mean age 4.34 years, control mean age 4.17 years). After serving as age-matched controls, five of the six age-matched control males (mean age 4.80 years) were paired with females and scanned seven days after pairing. All 12 paired males underwent a final PET scan approximately four months after being paired with a female (mean = 108.43 days, range = 91–119 days). The decision to pair all males following the one week timepoint, rather than leave them as control males until the last time point, was made for ethical reasons to avoid a long period alone for these males, and for practical reasons (for breeding) as this is the only laboratory breeding colony of titi monkeys in the world.

The choice of single housing as a baseline for this experiment was made for several reasons. There are few potential housing partners available for adult male titi monkeys, which cannot be housed with unrelated adult males due to aggression. We were also trying to avoid the presence of a current attachment bond in the adult males, which would happen if they were still in the family group with their natal attachment figure, their father. Therefore, males were housed singly for the unpaired control condition. At all times they had visual, auditory, and olfactory contact with other titi monkeys and were therefore still engaged in social communication. However, this choice of control should be taken into account in the interpretation of the results.

We also chose to conduct our social conditions sequentially rather than in a counter-balanced fashion. This was in part because we did not know if any effects caused by pairing would be reversible if pairs were split up, or if pairing would induce permanent changes.

For pair formation, one male and one female were introduced into their new home cage that was novel for both animals. The male was released into the cage first, immediately followed by the female. The male and female did not have visual access to each other prior to pairing.

PET Scanning with FDG

Subjects and female pair-mates were relocated to a metabolism room at CNPRC 48 hours prior to their positron emission tomography (PET) scan. As in our previous study (Bales et al, 2007), animals were relocated prior to the scan in order to reduce the possible effect of novel housing on brain metabolism. Animals were fasted 6–12 h prior to the scan, with water available throughout the pre-scan period. On the day of the PET scan, the male was caught and removed from the cage. The subject was manually restrained while he received a bolus injection of [^{18}F]-fluorodeoxyglucose (FDG, PETNET Solutions, Sacramento, CA) (mean = 1.979 mCi IV, SE = 0.04, 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 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). After the animal was sedated, a blood sample was collected from the femoral vein into a 3 ml heparin-coated tube and a sample of cerebrospinal fluid (CSF) was collected and put on ice. An endotracheal tube was placed and a catheter was placed in the saphenous vein in order to administer IV fluids (lactated ringers solution, 10 ml/kg/hr). Atipamazole was used to reverse medetomidine, and anesthesia was maintained with isoflurane (1–2%), while the male was positioned on the scanner bed feet first and the brain of the animal was positioned in the center of the scanner. PET imaging was performed on a microPET P4 scanner (Siemens Preclinical Solutions, Knoxville, TN). Image acquisition began a mean of 69.81 minutes (SE=1.41) post-FDG administration, and static PET scans were acquired for 60 minutes. Anesthesia was maintained throughout the scan. Animals were maintained in metabolism cages for 24 h after scanning, at which time radiation was decayed to background levels and animals were returned to their home cages.

MRI Scanning

Structural magnetic resonance imaging (MRI) scans were conducted in a GE Signa LX 9.1 scanner (General Electric Corporation, Milwaukee, WI) with a 1.5 T field strength and a 3″ surface coil. Each male was fasted 8–12 h before the procedure. At the start of the procedure, the male was sedated with ketamine (10 mg/kg IM) and medazolam (0.1 mg/kg IM), and an endotracheal tube was placed. A catheter was also placed in the saphenous vein in order to administer fluids as necessary. Anesthesia was maintained with isoflurane (1–2%) while the male was positioned in the MRI scanner. Each scan lasted approximately 20 min and consisted of a 3D SPGR pulse sequence in a coronal plane. Images of the entire brain were collected using the following parameters: echo time TE=7.9 ms, repetition time TR=22.0 ms, flip angle=30.0°, field of view=8 cm, number of excitations=3, matrix=256×256, and slice thickness=1 mm. As a precautionary measure, the male's EtCO2, oxygen saturation, heart rate and blood pressure were monitored throughout.

PET and MRI Coregistration, Quantification of FDG Uptake

The following regions of interest (ROIs) were examined: nucleus accumbens, medial amygdala, periaquaductal gray (PAG), cerebellum, putamen, caudate, prefrontal cortex, lateral septum, ventral pallidum, medial preoptic area (MPOA), supraoptic nucleus of the hypothalamus (SON), paraventricular nucleus of the hypothalamus (PVN), and posterior cingulate cortex (PCC) (Figure 2).

ROI structures including whole brain were drawn on each subject's MRI image using Siemen's Inveon Research Workplace software (IRW, Siemens Healthcare, USA). Static PET images were reconstructed with a 3DRP reconstruction protocol. Because the size of the head is similar in all subjects, and comparisons were made across the same structure in different animals, the PET data were not corrected for photon attenuation or scatter. Unlike rhesus macaque monkeys that have large heads, thick skulls and need transmission scans, titi monkeys have much smaller heads, thin skulls and therefore scatter and attenuation are similar to levels encountered in rodent studies and considered to be small effects. Correcting for attenuation or scatter would require an extra transmission scan, on another day and this would be logistically very difficult to acquire since we are measuring conscious uptake due to the unique social circumstances of each test day. 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 for the PET images were determined by applying ROIs defined on the MRI images to the PET images in IRW.

Typically, ROI mean FDG activities (in units of microcuries per cubic centimeter) are normalized by dividing by whole brain mean FDG activity (i.e. rCGM/GCGM). The resulting proportions of whole brain FDG uptake are used for statistical analysis, as was done in our previous study (Bales et al., 2007). In the current study, we were interested in changes in the "numerator" region of interest FDG uptake (rCGM) as well as the "denominator" whole brain FDG uptake (GCGM). Therefore, we corrected FDG uptake by the injected dose; this was done by dividing the ROI activity data by the injected dose and multiplying by 100%. This normalizes the FDG activity data for the amount injected into the subject, and is a standardized unit to use for statistical analysis. Data are presented in percent injected dose per gram of tissue (%ID/g) for rCGM regions of interest and for whole brain GCGM.

Blood sampling, timing and hormone analysis

Blood and CSF samples were collected after animals were sedated for the PET scan following the FDG uptake period, and placed on ice. In order to examine whether capture and sedation affected hormone concentrations, we collected timing data. We recorded what times blood and CSF samples were collected, and when animals were captured prior to their blood draw. Blood samples (n=43) were collected a mean of 5.32 min (SE=0.88) after capture and sedation of the subjects, and CSF samples (n=35) were collected a mean of 10.52 min (SE=0.37) after capture and sedation. While the veterinarians collected as many CSF samples as possible, sometimes they were unable to get a sample due to the small size of the animals (adults weigh approximately 1.0 – 1.5 kg). Blood samples in heparin-containing tubes were centrifuged at 3000 RPM for 15 minutes at 4° C. Plasma and CSF

samples were stored at −70° C until assay. Plasma samples were assayed for oxytocin (OT), vasopressin (AVP), cortisol, insulin and glucose. CSF samples were assayed for AVP and OT. There were no statistically significant relationships ($p>0.05$) between the amount of time taken to collect the blood or CSF sample ("disturbance time") and the hormone concentration of the sample.

AVP and OT concentrations were estimated in duplicate using commercial enzyme immunoassay kits (Enzo Life Sciences, Farmingdale, NY) previously validated for titi monkeys. Assay sensitivity was determined to be 2.34 pg/ml for AVP and 15.55 pg/ml for OT. Intra- and inter-assay coefficients of variation (CV) were 3.36% and 14.34% respectively for AVP, and 10.62% and 12.78%, respectively for OT. All CSF samples (n=35) were assayed for AVP. Thirty CSF samples were assayed for OT, because there were 5 samples that did not have sufficient volume to measure both hormones.

Plasma cortisol concentrations were estimated in duplicate using commercial radioimmunoassay kits (Siemens Healthcare, Malvern, PA), previously validated for titi monkeys (Mendoza, unpublished data) and has been used many times to analyze cortisol in this species (Hoffman et al., 1995, Bales et al., 2007, Jarcho et al., 2011, Laugero et al., 2011, Ragen et al., 2013). Prior to assay, samples were diluted 1:4 in PBS gel buffer. Assay procedures were modified with the addition of 0.5 and 2.35 μg/dl concentrations of standards along with the provided range of 1.0–49 μg/dl. Assay sensitivity has been determined to be 0.261 μg/dl. Intra- and inter-assay CV were 3.20% and 6.26%, respectively.

Plasma insulin concentrations were measured in duplicate using commercial ELISA kits (Ultra Sensitive Rat Insulin, Crystal Chem Inc., Downers Grove, IL) as per manufacturer's instructions for the wide range assay (0.1–12.8 ng/mL). This assay was validated for titi monkeys by assessing parallelism. All but one sample fell within the range of the assay, the lowest sample was set to the lowest assay standard (0.1 ng/ml). The antibody used in this insulin assay has high cross-reactivity with other species. Intra- and inter-assay CVs were 5.17% and 12.86%, respectively. Plasma glucose concentrations were measured in duplicate using commercial glucose colorimetric assay kits (Caymen Chemical Company, Ann Arbor, MI) as per manufacturer's instructions. This assay was validated for titi monkeys by assessing parallelism. All samples were in detectable range of the assay. Intra- and inter-assay CVs were 2.28% and 3.70%, respectively.

Data Analysis

In order to limit Type I error, we first grouped our ROIs into hypothesis-driven factors. These included:

Factor 1: Motivational areas; Nucleus accumbens, caudate, putamen, ventral pallidum

Factor 2: Social areas; Medial amygdala, lateral septum, posterior cingulate cortex, medial preoptic area

Factor 3: Control areas; Periaqueductal gray, cerebellum

Factor 4: Areas that produce OT and AVP; Paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus

Factor 5: Prefrontal cortex (PFC)

While there were many possible ways to make these groupings, we chose these on the following principles:

1. Dopaminergic areas of the dorsal and ventral striatum were grouped together in Factor 1 and labeled Motivational areas. All of these areas have been previously implicated in either rodent (Resendez et al., 2016) or human (Acevedo et al., 2012) studies of pair bonding/romantic love, or both.
2. Areas involved in social recognition and social memory. The medial amygdala and lateral septum are both involved in these processes (Engelmann and Landgraf, 1994, Noack et al., 2015). The posterior cingulate has been implicated in sexual fidelity and space use in monogamous voles (Ophir et al., 2008). The medial preoptic area, while better known for its role in maternal memory (Dobolyi et al., 2014, Stolzenberg and Champagne, 2016), has also been implicated in oxytocinergic regulation of social recognition (Popik and van Ree, 1991). All of these areas were also previously implicated in our cross-sectional study of pair bonding in male titi monkeys (Bales et al., 2007).
3. "Control" areas. We chose the periaqueductal gray as one of our control areas that was not expected to change with pair bonding because: a) there was no difference between pair bonded and non-bonded males in this area in our cross-sectional study (Bales et al., 2007); and b) in a direct comparison of romantic love and maternal love in humans, the periaqueductal gray was activated by maternal but not romantic love (Bartels and Zeki, 2004). We chose the cerebellum as a second control area, again due to the lack of a known association with pair bonding.
4. We grouped the areas of the brain that produce OT and AVP peptide, the PVN and SON.
5. Finally, we felt that the PFC did not group naturally with any of the other factors. However, the size of the medial PFC varies in rodent species with differing social systems (monogamous species having a smaller PFC) (Kingsbury et al., 2012). The PFC has also shown plasticity in primates with regard to other types of social behavior (Kozorovitskiy et al., 2006).

To examine the effects of pairing between the experimental and control groups over time, we carried out growth curve analyses and added group as an external variable to test for differences in intercept and slope between groups. Specifically, the model that we used can be expressed as

$$Y_{it}=y_{i0}+B_t \cdot y_{is}+e_{it}$$

where Y_{it} is the observed score on subject i at measurement t, y_{i0} is the initial level score of subject i, y_{is} is the slope, or the individual change over time, B_t is the set of coefficients that define the shape of the curve, and e_{it} is the error score of subject i at measurement t. Sources

of individual differences as well as the effects of a grouping variable on the level and slope can be included in the model as

$$y_{i0}=\gamma_0+\gamma_{0x}\cdot X_i+e_{i0} \text{ and}$$
$$y_{is}=\gamma_s+\gamma_{sx}\cdot X_i+e_{is}$$

where the level and slope scores now have fixed group intercepts (γ_0 and γ_s), and the regression coefficients (γ_{0x} and γ_{sx}), represent the effect of the observed variable X on the level and slope. This model follows the same formulation as hierarchical, multilevel, or random-effects models (Laird and Ware, 1982, Bryk and Raudenbush, 1992, McArdle et al., 2002, Ferrer et al., 2004).

In our analyses, we considered two sets of coefficients B_t to denote the shape of the curve. In the first set, we used B_t = [0,1,2] to represent a linear model of change across the three measurement occasions. In the second set, we used B_t = [0,1,2], including a latent basis for determining nonlinearity in the changes across the three occasions. Finally, to facilitate the interpretation of parameters, group was centered-coded (−1 = control; +1 = experimental).

Results

Results from these analyses are presented in Figure 1 and Table 1 for the key neural variables (representing regional cerebral glucose metabolism) in our study. For example, for *factor 1 (Motivational areas)* in Table 1, the results of the linear model indicate an average intercept (scores at baseline; y_0 = .106), and an average slope (y_s =.013), representing linear changes per measurement occasions. Both coefficients were reliably different from zero. The group effect on the intercept was not statistically significant (γ_{0x} = −.005) indicating that both groups did not differ at baseline. However, the group effect on the slope was different from zero (γ_{sx} = .016), indicating that the experimental group had a larger slope than the control group. Finally, about 54% of the variance in this *factor 1* was explained by the time and group effects.

The results from the nonlinear model are similar but consider an additional parameter. These results indicate an average intercept (scores at baseline; y_0 = .106) an average slope (y_s =. 025), representing the total change from baseline to the last occasion, and a latent basis (B_2 = .593), indicating that about 59% of all the changes took place from baseline to one week post-pairing. As was the case for the linear model, the group effect on the intercept was not statistically significant (γ_{0x} = −.006) indicating that both groups did not differ at baseline. As before, the group effect on the slope was different from zero (γ_{sx} = .032), indicating that the experimental group showed more total changes than the control group. This model including nonlinear time and group effects explained about 60% of the variance in *factor 1*.

Table 1 shows that for all five neural factors, the groups do not differ at baseline in both linear and nonlinear models. The group effect on the slope is significantly different from zero for all five factors in both linear and non-linear models, indicating that for all five factors the experimental group changed more than the control group. The latent basis for all

five factors varies from .593 for *factor 1*, to .814 for *factor 4*. Thus for all five factors, a majority of the change in the experimental group occurred in the first week post-pairing. For the PVN and SON, over 81% of the change occurred in the first week post-pairing.

Values in Table 1 are based on data normalized for injected dose and are in units of % injected dose/gram (%ID/g), rather than on data normalized for GCGM (i.e. rCGM/GCGM) where units would be proportion of whole brain FDG uptake (proportion of GCGM). In Table 2 we show that for GCGM, the group effect on the slope was different from zero (γ_{sx} =.014), indicating that experimental animals had a greater change in GCGM than the control group. The % variance explained by the linear model is 42.3%.

We were also interested in how much of the change in rCGM for each factor can be explained by the changes in GCGM. For a linear model, Factors 1 (Motivational areas) and 2 (Social areas) explained approximately 10% more variance than GCGM (53.6% and 52.1%, respectively, vs. 42.3%). Factor 3 (control areas) explain only slightly more variation (6%) than GCGM at 48.3%. Factors 4 and 5 both explain less variance than explained by the change in GCGM. For the non-linear model, most of the relationships are similar. However, with GCGM explaining 57.8% of variance in a non-linear model, Factor 1 does not improve the model with 59.7% explained.

Table 3 shows the models for the hormonal variables: Plasma insulin, glucose, cortisol, OT, and AVP; as well as CSF OT and AVP. Baselines did not differ between groups for any of the hormones examined. No changes in hormonal concentrations were significantly different between the experimental group and the control group. Plasma insulin displayed a significant negative slope over time (γ_{sx} =−.785).

Discussion

These results confirm, in a longitudinal model with an age-matched control group, that titi monkey males show an increase in whole brain neural FDG uptake within one week of pairing. Within the brain, these increases are more marked in areas rich in dopamine receptors (“Motivationalal”) and areas involved in the formation of social memories. These increases are still evident, and even exaggerated, after four months post-pairing.

This study focused primarily on hypothesis-driven examination of forebrain neural areas suggested by studies in prairie voles and our own previous cross-sectional study. Measurement of FDG uptake is not specific as far as what type of peptide release or receptor activation is occurring in each area, although the assumption was that similar peptides would be involved as are in voles (primarily AVP and OT). Our finding of increased rCGM in areas of the mesolimbocortical system (NAcc, VP) strongly suggests the involvement of dopamine and opioids in this process. The formation of a pair bond is likely to be highly motivated both because of the reward value involved with mating (Pfaus et al., 2012) as well as the social reward (Northcutt and Lonstein, 2009). However, based on our previous findings that dopamine D1 receptors did not change in the NAcc and VP with pair-bonding in male titi monkeys, it is more likely that D2 receptors were involved in these areas (Hostetler et al., 2016). Also likely involved are μ and κ opioid receptors, which are involved in pair bond

regulation in both prairie voles (Resendez et al., 2012, Resendez et al., 2013) and titi monkeys (Ragen et al., 2013, Ragen et al., 2015b). Opioid receptors in titi monkeys are found in primarily the same areas as in other primates, throughout the forebrain including many of the areas we included in factors 1 and 2 (Ragen et al., 2015a).

This study did not support the hypothesis that sustained activation of OT and AVP *producing* areas occurs during pair bond formation and maintenance in male titi monkeys, although it does not eliminate the possibility that FDG uptake in these areas (SON and PVN) is increased on a shorter timescale (for instance, within 48 hours of pairing as seen in (Bales et al., 2007)). Indeed, the non-linear model showed that over 80% of the change in these areas occurred in the first week post-pairing, which was substantially higher than for other factors. The question of whether areas with OT and V1a receptors, rather than peptide, are involved is a more complicated one. We recently published the OT and V1a receptor distribution of titi monkeys. In contrast to the more common biomedical model, the rhesus monkey (*Macaca mulatta*) (Freeman et al., 2014a), titi monkeys display OT receptors in more forebrain areas, including the lateral septum (Freeman et al., 2014b). However, given that V1a receptors are much more widespread than OT receptors even in titi monkeys, their involvement is very likely. In particular, the titi monkey has V1a rather than OT receptors in the NAcc, which was implicated in this study.

This study did not find any changes in plasma hormone concentrations of AVP, OT, cortisol, glucose or insulin in response to pair formation or maintenance from blood samples collected on PET scan days. Similarly, there were no changes in CSF AVP or OT in response to pairing. However, the many events that happened on blood sampling day (i.e. housing in new cage and room, captured twice, sedation) could easily have obscured sensitive hormonal changes. Ideally, “baseline blood samples” are collected when animals are housed in their home cages and animals can be captured quickly and blood can be collected before the stress of capture affects the hormonal concentrations (Mendoza, 2017). Timing of handling and blood sampling in this study was optimized for the PET procedure rather than measurement of hormones. We did find that subjects showed a decrease in plasma insulin concentrations over time, perhaps as a response to habituation to the procedures.

A major finding of this study was the long-term increase in brain glucose metabolism due to pairing. While it might be thought at first that this was due to an impoverished social state prior to pairing, we do not believe this to be the case; all males had visual, olfactory and auditory access to multiple other groups of titi monkeys. An associated shift was not seen in plasma glucose concentrations with pairing in this sample. Most studies of FDG uptake use data that are normalized by using proportions of whole brain activity, which is calculated by dividing the regional cerebral glucose metabolism (rCGM) by the GCGM. Thus, in many cases group differences in GCGM are not examined, reported and are assumed to not exist. Exceptions include aging (Kochunov et al., 2009), Alzheimer’s disease (Cunnane et al., 2011), exercise (Kemppainen et al., 2005), and traumatic brain injury (Soustiel et al., 2005), all of which result in a *reduction* in GCGM. To our knowledge, establishment of a pair bond may be one of the only non-pharmacological manipulations to lead to long-term increases in whole brain FDG uptake in an adult.

There are still many unanswered questions in the neurobiology of social bonds in titi monkeys and other primate species. It is unclear if whole brain FDG uptake would increase further more than four months post pairing, and whether this increase would be sustained indefinitely. The neurochemicals which are responsible for the higher rCGM in some regions remain to be confirmed. Chemically specific PET ligands are available for some of these (such as dopamine D1 and D2 receptors); however, there is still no OT receptor PET ligand (Smith et al., 2016), and no easily available V1a receptor PET ligand. Finally, the neural basis of other aspects of the pair-bond, such as separation distress and mate-guarding (or "jealousy"), also remain to be considered.

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Highlights

- Socially monogamous male titi monkeys were followed longitudinally during pair bond formation.
- Paired males displayed an increase in global cerebral glucose metabolism until at least four months post pairing.
- Areas involved in motivation and in social memory displayed higher increases than the whole brain increases.

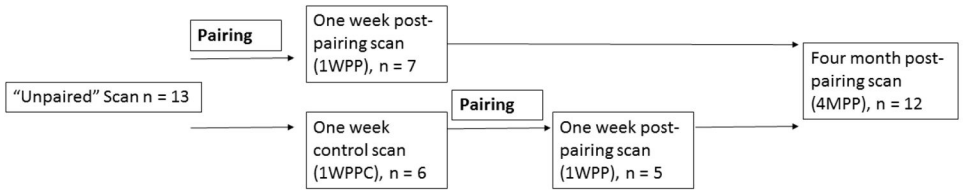

Figure 1.

Experimental design of the project. At baseline ("Unpaired"), experimental and control groups were not paired and did not differ on any demographic or outcome measures. The experimental group was then paired, and outcome measures collected at one week post-pairing (1WPP). The control group was still housed alone at this point (1WPPC). The control group was then also paired and provided a one week post-pairing timepoint as well. All males were rescanned at four months post-pairing (4MPP).

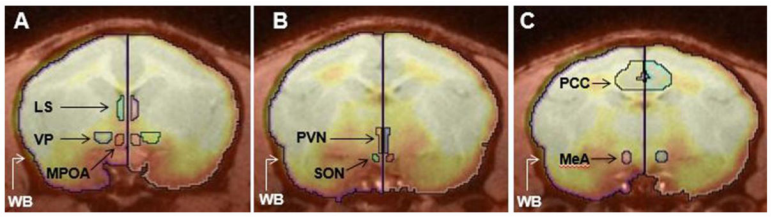

Figure 2.

Positron emission tomography (PET) image of a titi monkey brain co-registered with magnetic resonance imaging (MRI) with regions of interest outlined in Inveon Research Workplace (IRW) software. Panel A shows the coronal view of the lateral septum (LS), ventral pallidum (VP), and medial preoptic area (MPOA). Panel B shows the coronal view of the paraventricular nucleus of the hypothalamus (PVN) and the supraoptic nuclues of the hypothalamus (SON). Panel C shows the coronal view of the posterior cingulate cortex (PCC) and medial amygdala (MeA). All three panels show the outline of the Whole Brain (WB).

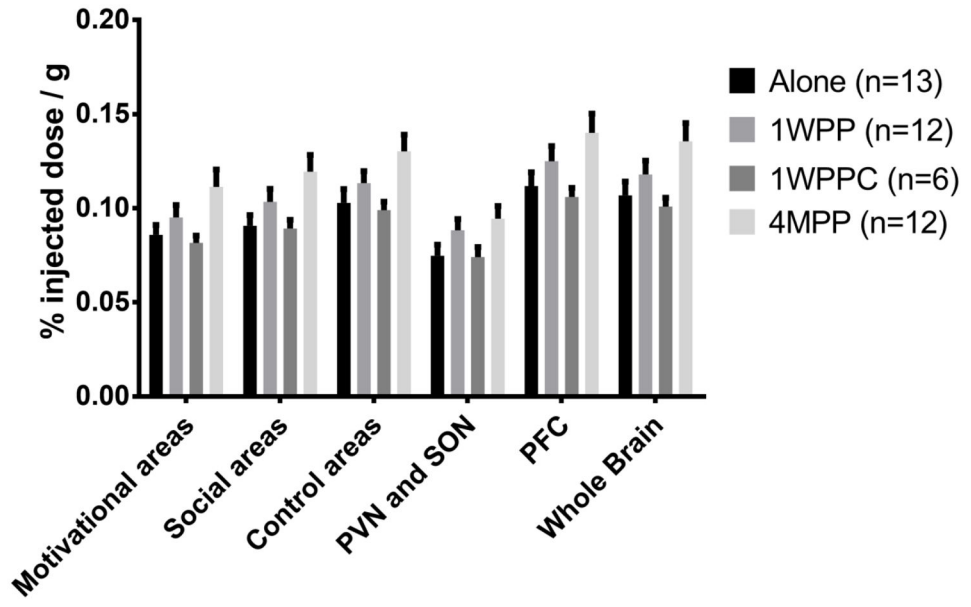

Figure 3.

FDG uptake, in units of % injected dose/g, for factors including Motivational areas included in Factor 1 (NAcc, VP, Caud, Put); social areas included in Factor 2 (MeA, LS, MPOA, PCC); control areas included in Factor 3 (PAG, Cere); PVN and SON (Factor 4); PFC (Factor 5), during Alone PET scan (Alone), one week post-pairing scan (1WPP), one week post-pairing control scan (1WPPC), and four months post-pairing scan (4MPP). All factors showed a significant effect of experimental group in a linear model. However, Factors 1 and 2 showed greater change than GCGM.

Table 1

Parameter Estimates from Curve Models with Group Differences for Factors

Parameters	**Factor 1: Motivational Areas**	**Factor 2: Social Areas**	**Factor 3: Control Areas**	**Factor 4: PVN and SON**	**Factor 5: PFC**
Linear Model					
Intercept	.106 (.005)*	.091 (.005)*	.102 (.006)*	.076 (.005)*	.111 (.006)*
Time slope[a]	.013 (.006)*	.012 (.005)*	.011 (.006)*	.009 (.004)*	.012 (.006)*
Group*Intercept	−.004 (.005)n	−.003 (.005)n	−.006 (.006)n	−.001 (.005)n	−.005 (.006)n
Group*Slope	.016 (.006)*	.012 (.005)*	.013 (.006)*	.008 (.004)*	.015 (.006)*
Residual Variance	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*
−2LL	172.5	186.3	179.4	188.9	173.0
BIC	154.5	168.3	158.9	171.0	155.0
% Variance[d]	.536	.521	.483	.326	.328
Nonlinear Model					
Intercept	.106 (.006)*	.090 (.005)*	.102 (.006)*	.075 (.006)*	.111 (.006)*
Time slope[b]	.025 (.012)n	.023 (.010)*	.022 (.011)n	.016 (.008)*	.022 (.011)n
Latent basis[c]	.593 (.151)*	.624 (.150)*	.621 (.157)*	.851 (.234)*	.676 (.200)*
Group*Intercept	−.006 (.006)n	−.005 (.005)n	−.007 (.006)n	−.003 (.006)n	−.007 (.007)n
Group*Slope	.032 (.012)*	.025 (.010)*	.027 (.011)*	.017 (.008)*	.032 (.011)*
Residual Variance	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*	<.001 (<.001)*
−2LL	172.9	186.9	179.9	190.9	173.9
BIC	149.9	163.9	156.8	167.8	150.9
% Variance[d]	.597	.625	.618	.479	.512

Note. Entries are parameter estimates and standard errors (in parentheses).

*parameter estimate with $p < .05$.

*n*parameter estimate with $p > .05$.

Intercept = scores at baseline.

[a]changes per measurement occasion.

[b] total changes from baseline to four months post pairing.

[c] percentage of changes from the baseline to 7-days post pairing.

[d] Explained variance due to time and group effects.

Number of subjects = 13; number of observations = 43. Group code (−1 = control; +1 = experimental). Factor 1 includes Motivational areas (NAcc, VP, Caud, Put); Factor 2 includes social areas (MeA, LS, MPOA, PCC); Factor 3 includes control areas (PAG, Cere); Factor 4 includes OT and AVP producing nuclei (PVN, SON); Factor 5 is the prefrontal cortex (PFC).

Table 2

Parameter Estimates from Curve Models with Group Differences for Whole Brain

Parameters	Whole Brain
Linear Model	
Intercept	.106 (.006)*
Time slope[a]	.012 (.006)*
Group * Intercept	−.004 (.006)[n]
Group * Slope	.014 (.006)*
Residual Variance	<.001 (<.001)*
−2LL	176.9
BIC	158.9
% Variance[d]	.423
Nonlinear Model	
Intercept	.106 (.006)*
Time slope[b]	.023 (.011)*
Latent basis[c]	.631 (.168)*
Group * Intercept	−.006 (.006)[n]
Group * Slope	.029 (.011)*
Residual Variance	<.001 (<.001)*
−2LL	177.4
BIC	154.3
% Variance[d]	.578

Note. Entries are parameter estimates and standard errors (in parentheses).

* parameter estimate with $p < .05$.

[n] parameter estimate with $p > .05$.

Intercept = scores at baseline.

[a] changes per measurement occasion.

[b] total changes from baseline to the four month post-pairing timepoint.

[c] percentage of changes from the baseline to 7-days post pairing.

[d] Explained variance due to time and group effects.

Number of subjects = 13; number of observations = 43. Group code (−1 = control; +1 = experimental).

Table 3

Parameter Estimates from Curve Models with Group Differences for Hormonal Variables

Parameters	CSF OT	Plasma OT	CSF AVP	Plasma AVP	Plasma Insulin	Plasma Glucose	Plasma Cortisol
Linear Model							
Intercept	48.7 (5.75)*	711.3 (41.4)*	3.55 (.664)*	291.9 (18.6)*	2.31 (.623)*	93.4 (5.39)*	90.9 (6.32)*
Time slope[a]	4.57 (4.29)n	−10.2 (22.3)n	−.092 (.611)n	11.6 (14.9)n	−.785 (.280)*	1.62 (2.47)n	−5.88 (3.24)n
Group * Intercept	.056 (5.75)n	−54.6 (41.4)n	−1.11 (.664)n	−9.29 (18.7)n	−.588 (.623)n	.038 (5.39)n	−9.99 (6.31)n
Group * Slope	−.168 (4.29)n	18.4 (22.3)n	.996 (.611)n	13.1 (14.9)n	.370 (.280)n	−3.43 (2.47)n	2.99 (3.24)n
Residual Variance	279 (110)*	11593 (3340)*	2.94 (1.46)*	5235 (1516)*	.744 (.304)*	146.9 (42.0)*	147.4 (60.1)*
−2LL	213.6	472.8	122.7	431.9	108.5	310.7	320.4
BIC	231.6	490.8	143.2	449.9	129.0	328.6	340.9
% Variance[d]	.114	.032	.309	.060	.596	.071	.375

Note. Entries are parameter estimates and standard errors (in parentheses).

*parameter estimate with $p < .05$.

*n*parameter estimate with $p > .05$.

Intercept = scores at baseline.

*a*changes per measurement occasion.

*d*Explained variance due to time and group effects.

Number of subjects = 13; number of observations = 43 for plasma hormones; 30 for CSF OT and 35 for CSF AVP. Group code (−1 = control; +1 = experimental).