Published in final edited form as:
Brain Res. 2007 December 12; 1184: 245–253.

Neural Correlates of Pair-bonding in a Monogamous Primate

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Abstract

The neurobiology of social bonding, despite its relevance to human mental health, has been studied primarily in rodents. In this study we used position emission tomography (PET), registered with structural magnetic resonance imaging (MRI) to investigate central glucose uptake in seventeen males of a monogamous primate species, the titi monkey (*Callicebus cupreus*). Twelve pair-bonded males (including six with a lesion of the prefrontal cortex) and five lone males were scanned. The five lone males were re-scanned 48 hours after pairing with a female. Significant differences in glucose uptake were found between males in long-term pair-bonds and lone males in areas including the nucleus accumbens, ventral pallidum, medial preoptic area, medial amygdala, and the supraoptic nucleus of the hypothalamus. In paired before and after comparisons, males showed significant changes following pairing in the right nucleus accumbens and ventral pallidum but not in other areas. Lesioned males showed significantly higher uptake in the posterior cingulate cortex than all other males. These results indicate some basic similarities between rodents and primates in the formation and maintenance of selective social bonds, but emphasize the importance of studying long-term maintenance in addition to short-term formation of social bonds.

Keywords

social bonding; monogamy; oxytocin; vasopressin

1. Introduction

The neurobiology of pair-bonding has been well-studied in rodent models, particularly the monogamous prairie vole (Winslow et al., 1993;Williams et al., 1994;Carter, 1998;Insel et al., 1998;Cho et al., 1999;Lim et al., 2001;Young et al., 2001a;Aragona and Wang, 2004). This work in rodents has identified a neural circuit beginning with sensory input into the olfactory system, and involving both the “reward circuit” (ventral pallidum, nucleus accumbens, ventral tegmental area) and the “social recognition” circuits in the medial amygdala and lateral septum (Liu et al., 2001;Young et al., 2001b;Lim et al., 2004a;Young et al., 2005). The critical areas for the formation of pair-bonds are hypothesized to be the nucleus accumbens in females (Aragona et al., 2005) and the ventral pallidum in males (Lim et al., 2001;Lim and Young, 2004;Lim et al., 2004b). A recent model of affiliation in humans also concentrated on the μ-

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opioid receptors in the nucleus accumbens as mediators of the experience of affiliative reward (Depue and Morrone-Strupinsky, 2005).

The neurobiological basis of social bonds in humans has become an important topic in recent years, particularly with the increasing incidence of disorders in social bonding such as autism (Insel et al., 1999;Lim et al., 2005). Some very interesting human studies have used fMRI to investigate areas activated or deactivated while viewing objects of attachment (Bartels and Zeki, 2000;Bartels and Zeki, 2004;Aron et al., 2005;Fisher et al., 2005;Fisher et al., 2006). These studies have identified some of the same brain regions of importance noted in the animal studies, including areas in the hypothalamus and the reward circuit. Animal studies have the advantage, of course, of offering a simpler, perhaps less variable model system. In studying titi monkeys, we can manipulate the exact time and conditions of the formation of a pair-bond. There is also the potential to administer experimental treatments which may affect the quality of the pair-bond, or the speed of its formation.

It is worth noting, however, that almost all previous research on the neurobiology of pair-bonds in animals has been done on rodents, and almost exclusively on the *formation* rather than on the *maintenance* of the bond. In the classic partner preference paradigm (Williams et al., 1992), a vole or other rodent chooses between a cage containing a partner, a cage containing a stranger, or an empty cage. This is almost always carried out in the context of giving a treatment followed by a short cohabitation period, and then a test for presence of a bond. It has rarely been carried out on established pairs.

Research on the neurobiology of either formation or maintenance of pair-bonds in non-human primates, the mammals most closely related to humans, is relatively meager. Partly this is because the common laboratory biomedical primate model, the rhesus macaque (*Macaca mulatta*) does not form pair-bonds. Rhesus monkey sexual consortships have been used as a model of sexual jealousy (Rilling et al., 2004), but these are temporary associations Monogamous primates represent relatively few species, including the lesser apes (such as gibbons and siamangs), and New World monkeys such as marmosets, tamarins, saki monkeys, owl monkeys, and titi monkeys (Kleiman, 1977).

In the present research, we used monogamous titi monkeys (*Callicebu scupreus*). These small, arboreal South American monkeys form strong emotional bonds between pair-mates (Mendoza and Mason, 1997). In the wild and in captivity, this bond is reflected in the close coordination of travel between pair-mates, and the large amount of time they spend sitting in physical contact with their tails twined (Mason, 1968). Separation from the pair-mate produces a sustained rise in glucocorticoids, even if the remaining pair-mate is still in a familiar environment and with other familiar animals (Mason and Mendoza, 1998).

This study was intended to identify those brain areas which vary in metabolic rate for glucose uptake in males in different types of social bonds; in particular, those in long-term pair-bonds vs. newly-formed bonds (maintenance vs. formation of bonds), vs. those which were not yet paired. A subset of males had also been part of a previous lesioning experiment (see experimental procedures), which resulted behaviorally in increased interaction with their pair-mates and apparent “strengthening” of the pair-bond. Specifically, post-lesion the males were more often in physical contact with their mates, were less likely to break contact, and spent more time grooming their mates. They also displayed less behavioral arousal (arching, tail-lashing) towards strange females than they did pre-lesion (Mendoza et al., 2006); Mendoza et al., submitted). They thus provide an interesting comparison group which displays an apparently up-regulated pair-bond.

We used a combination of positron emission tomography (PET) and structural MRI (Figure 1 and Figure 2) in order to determine glucose uptake in different neural regions in these groups

of males, predicting that we would see differing uptake in areas similar to those seen in rodents [nucleus accumbens (Nacc), ventral pallidum (VP), medial preoptic area (MPOA), medial amygdala (MeA), supraoptic nucleus (SON), paraventricular nucleus (PVN), lateral septum (LS), and the posterior cingulate cortex (PCg)]. In addition, we predicted that these differences would be more apparent in long-term pairs than in newly-formed pairs. Control areas included: 1) the caudate-putamen (CP). While subareas of the CP have been implicated in human studies (Bartels and Zeki, 2000;Fisher et al., 2005), the dorsal striatum has not been implicated in rodent studies, which have focused on the ventral striatum. In contrast, the dorsal striatum is usually examined in relation to locomotion; 2) the central amygdala (CeA), which has both OT and AVP receptors but is more classically associated with fear than affiliation; and 3) the periaqueductal gray (PAG), which was activated in human studies by maternal but not romantic love (Bartels and Zeki, 2004) and is associated with nursing behavior in rodents (Lonstein and Stern, 1997). Clearly these three areas have functions other than those mentioned here, but based on rodent studies were not predicted to be involved in pair-bonding.

2. Results

Glucose uptake, long-term paired males, lesioned males, and lone males

An overall MANOVA for glucose uptake, with hemisphere as a repeated measures variable, was significant for bonding status (Wilk's lambda = 0.167, $F_{16,42} = 3.79$, $p = 0.0003$) but not for hemisphere (Wilk's lambda = 0.641, $F_{8,21} = 1.47$, $p = 0.226$) or a status by hemisphere interaction (Wilk's lambda = 0.652, $F_{16,42} = 0.62$, $p = 0.854$).

Individual ANOVAs (Figure 3) showed that uptake differed significantly by bonding status in the nucleus accumbens ($F_2 = 7.63$, $p = 0.0023$), ventral pallidum ($F_2 = 6.39$, $p = 0.0052$), medial preoptic area ($F_2 = 9.05$, $p = 0.0009$), the medial amygdala ($F_2 = 7.74$, $p = 0.0021$), supraoptic nucleus ($F_2 = 13.51$, $p < 0.0001$), lateral septum ($F_2 = 8.38$, $p = 0.0014$), and posterior cingulate cortex ($F_2 = 8.52$, $p = 0.0016$). Uptake did not differ in the paraventricular nucleus of the hypothalamus ($F_2 = 2.48$, $p = 0.102$).

In the posterior cingulate cortex, post-hoc tests indicated that the lesioned males had significantly higher glucose uptake than the pair-bonded and lone males. In all other areas with significant ANOVAs, the lone males differed significantly from both pair-bonded and lesioned males, while these two groups did not differ from each other.

Control areas—A MANOVA performed on control areas (CP, CeA, PAG; Figure 4) not predicted to be involved in pair-bonding did not find significant differences by bonding status (Wilk's lambda = 0.75, $F_{6,52} = 1.34$, $p = 0.256$), hemisphere (Wilk's lambda = 0.972, $F_{3,26} = 0.25$, $p = 0.858$), or a hemisphere by status interaction (Wilk's lambda = 0.933, $F_{6,52} = 0.30$, $p = 0.932$).

Glucose uptake: changes after pairing

Because of the small number of newly paired males (n = 5), we were not able to run models assessing hemisphere and status by hemisphere interactions; we therefore ran paired tests for areas on the left and right hemispheres separately. When males were compared before and after pairing, both the nucleus accumbens ($t_4 = 2.86$, $p = 0.023$, one-tailed; Table 2) and the ventral pallidum ($t_4 = 2.30$, $p = 0.042$, one-tailed) showed significant changes in glucose uptake in the right hemisphere of the brain. No neural areas in the left hemisphere showed differences before and shortly after pairing.

Cortisol—Cortisol taken at the time of FDG injection did not differ by bonding status between paired, lesioned, and lone males ($F_{2,14} = 0.08$, $p = 0.93$) or in paired tests before and after

mating ($t_4 = -0.12$, $p = 0.907$). Means ± standard errors were: paired males, 51.39 ± 0.14 μg/dl; lesioned males, 50.48 ± 0.21 μg/dl; lone males, 49.36 ± 0.14 μg/dl; newly paired males, 50.82 ± 0.15 μg/dl.

3. Discussion

These results indicate significant differences in baseline regional glucose uptake between males in long-term pair-bonds vs. males which are not pair-bonded. In most cases, the areas in which uptake differed by bonding status were the same as those implicated in rodent studies. They also point to potentially important differences in central glucose uptake between the formation of a pair-bond and its maintenance. Males in long-term pair-bonds had significantly lower relative uptake in the Nacc, VP, MPOA, MeA, SON, and LS than lone males or newly paired males. Each of these areas has been implicated in rodent studies of pair-bonding and sexual activity and is part of either the reward system or an area with high levels of neuropeptide (oxytocin and vasopressin) production or receptors (which have in turn been implicated in reward and pair-bonding).

We examined both the Nacc and VP, both of which are rich in dopamine receptors and heavily involved in the neural control of incentive motivation (Berridge and Robinson, 2003;Depue and Morrone-Strupinsky, 2005). The Nacc is important in the formation of pair-bonds in both male and female voles (Aragona et al., 2003). The VP, however, has been spoken of as the critical area for pair-bonding in males, due to the co-localization of both vasopressin V1a receptors and D2 receptors (Lim and Young, 2004). Both the VP and the Nacc showed reduced relative glucose uptake in long-term paired males in our study. While it is difficult to know whether a reduction in glucose uptake represents a reduction of uptake by inhibitory or excitatory neurons, it is important to note that in both of these areas, new pair-bond formation significantly reduced relative glucose uptake; thus moving it in the direction of the males in longer-term pair-bonds (Table 2).

In addition to the reward circuit, areas strongly related to neuropeptide production or with high levels of receptors were implicated in the maintenance of pair-bonds. The SON is a primary area of production of OT and vasopressin (AVP), which are exported to the posterior pituitary via the median eminence, and thus to the rest of the body (Argiolas and Gessa, 1991;Barberis and Tribollet, 1996;Gimpl and Fahrenholz, 2001). Areas crucial to social memory such as the LS and MeA, which contain OT and AVP receptors, were also shown to differ by pairing status, with lower glucose uptake in long-term paired males. In some species including the monogamous prairie vole (De Vries and Villalba, 1997;De Vries and Miller, 1998) and marmoset (Wang et al., 1997), these areas are part of an extrahypothalamic, androgen-dependent vasopressin circuit. These areas are thought to be responsible for “social memory” (recognition and memory of individuals), which is crucial to the formation of long-term social bonds. This process appears to be mediated by AVP (V1a subtype) receptors, which when blocked or otherwise disrupted in the LS, eliminates social memory (Engelmann et al., 1996). As predicted, none of the control areas showed a difference in glucose uptake by pairing status.

Changes in glucose uptake in newly paired males showed a great deal of variability (Table 2) and only a few consistent changes. It is possible that this variability is due to individual differences in the process of forming a new bond. It is also interesting to note that whereas experimental pairing of a titi monkey male and female almost always results in a demonstrable pair-bond, the initial behavior upon being placed together can differ substantially. Approximately 10 hours of behavioral data were collected on each of the new pairs during the 48 hours between pairing and scanning. While one pair copulated after 17 minutes and two other pairs were tail-twining (an affiliative behavior) within a few hours, the final two pairs

were still spending almost all of their time on opposite sides of a partitioned cage after the full 48 hours. These variations in behavior could lead to interesting differences in neural activity, and could serve as a basis for studies of individual differences in the formation of social bonds (Depue and Morrone- Strupinsky, 2005).

It is possible that the differences we observed in glucose uptake were associated with age rather than bonding status, as most of our lone males were younger than our paired and lesioned males. However, we have several reasons to question this interpretation. First, the data are presented here as normalized units, which is to say that the mean VOI values are divided by whole-brain VOI value, so that glucose uptake for an area is *relative* to glucose uptake for the whole brain. By this measure, glucose uptake is lower in many areas for the paired and lesioned (i.e. the older) males. However, this method of calculation does not reflect the fact that *absolute* or whole-brain values were actually *higher* in paired and lesioned males than in the lone males ($F_2 = 4.99$, $p = 0.027$), an effect that is *opposite* to that you would expect based on normal changes in brain metabolism with age reported in the human imaging literature (Martin et al., 1991;Takada et al., 1992; Burns and Tyrrell, 1992;Zuendorf et al., 2003;Beason-Held et al., 2006;Kalpouzos et al., 2007).

We believe that bonding differences are the most reasonable interpretation of the data. However, if the changes that we report in glucose uptake can be ultimately attributed to age differences between the groups, it is still of considerable interest to the neurobiology of sociality since changes were restricted to areas previously associated with social bonding. We have long known that changes in psychosocial motivation occur from young to mature adulthood (Erikson, 1968). If aging accounts for the pattern of results obtained, then it stands to reason that we have identified the areas of the brain most likely to account for such an individual shift.

The only area in which lesioned males were significantly different from every other group was the PCg. This area is of great interest because of its association not only with “friendship” in human studies of imaging (Bartels and Zeki, 2004), but also its association with the monitoring of action-reward outcomes (Tabuchi et al., 2005). In addition, this area shows a large amount of variation in levels of OT and AVP receptors in certain species such as prairie voles (Phelps and Young, 2003). It is possible that the prefrontal lesion released the PCg from inhibition and led to the observed increase in affiliative behavior (Knight et al., 1999).

Despite an abiding psychological interest in human attachment (Bowlby, 1969), the neurobiological substrates of social bonding in humans are not well-understood, and in this area primate models may be especially important. For instance, in human formation of social bonds we might expect a much larger contribution of cortical areas than found in rodents, and in this study we did find a significant effect on the posterior cingulate cortex. Use of a non-human primate provides a laboratory model whose neuroanatomy is much closer to the human, and provides a link between rodent models and humans.

4. 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 seventeen captive-born adult male titi monkeys (*Callicebus cupreus*) housed at the California National Primate Research Center, Davis, CA. Animals were fed twice daily (08:30 and 13:30) a diet consisting of monkey chow, cottage cheese, marmoset jelly, apples,

raisins, baby carrots, and vitamins. Further details of husbandry and training are available elsewhere (Tardif et al., 2006) with caging identical to that described in (Mendoza, 1999).

The main independent variable in this study was whether or not the male was in a pair-bond, his "bonding status" Twelve of the seventeen males were maintained in family groups with a mate and offspring (Table 1). Six of the twelve males that were living with their mates had been part of a previous experiment (approximately one year previously) in which they had received a small bilateral lesion in the area of the prefrontal cortex defined by reciprocal projections from the somatosensory cortex ("Lesioned" group) (Padberg et al., 2005;Padberg and Krubitzer, 2006). Subsequent to the lesion, these six males increased their time in proximity to their mate (results of lesion further described in the introduction to this paper). Lesions were verified as to location via the structural MRIs collected for this study. In addition, the MRI slices containing the lesions were compared to equivalent slices in eleven non-lesioned males, and quantitative analysis via ImageJ (NIH, Bethesda, MD) indicated a 3.0 % reduction in prefrontal volume in the lesioned males. None of the neural areas examined in this study changed in size as a result of the lesions. The other six (non-lesioned) males living with their mates were designated as the "Paired" group.

The remaining five of the seventeen males were being housed individually at the start of this research for reasons of colony management. As a matter of practice, they are housed in this way until a suitable female becomes available. This group was designated as the "Lone" group. The five "lone" males, subsequent to their initial PET scan, were paired with females and re-scanned 48 hours later.

It should be noted that "Lone" males are housed in the same large colony room as the other monkeys, and thus have visual, auditory, and olfactory exposure to conspecifics. Daily checks are carried out to monitor health and appetite. Individually housed titi monkeys do not display any stereotypies, depressive-like behaviors, or impairment of health. They do display chronically elevated levels of cortisol (Mendoza et al., 2000), but it should be noted that at the time of the scan, cortisol levels did not differ between groups (see Results), indicating that differences in glucose uptake were not due to higher stress in one group of monkeys. In addition, ONLY the presence of an attachment figure reduces this elevated cortisol (Mendoza et al., 2000), not the presence of any other monkey (including a sibling). The effects of this housing therefore appear to be a specific response to lack of a pair-bond or other attachment, rather than a generalized response to living alone. Finally, in the wild there is some evidence that titi monkey males go through a "bachelor" period in which they range alone (Bossuyt, unpublished data). There is thus support for this housing arrangement as reflecting a normal aspect of natural history of the species.

PET scanning

Paired, lesioned, and lone males were scanned in balanced order (2–3 per scan date). For approximately 48 hours prior to PET scanning, the subject was housed in the metabolism room at the Primate Center (along with the mate and infants under one year old). This was done to reduce the effect of novel housing on brain metabolism. It is worth noting that male titi monkeys (though competent fathers) do not form an emotional attachment to their infants, show no distress or rise in cortisol upon separation from the infant, and do not distinguish their infants from infants of other groups (Mendoza and Mason, 1986;Mendoza and Mason, 1997). It is therefore probable that the additional presence of the infant did not greatly affect neural activity in the male.

Males were fasted for 8–12 h prior to the scan, with water available throughout the pre-scan period. On the day of the study, a blood sample was collected for measurement of cortisol. The subject then was manually restrained while it received a bolus [^{18}F]fluoro-2-deoxy-D-glucose

(FDG) injection (up to 1 mCi/kg i.v., administered in a volume of < 2 ml) over 30 sec into the saphenous vein. For the next 30 min, the subject was returned to its cage (with the mate, for males in the paired or lesioned groups). After 30 min, the animal was sedated with Ketamine anesthesia (5–30 mg/kg subcutaneous) and prepared for anesthesia. Anesthesia was induced with isoflurane (1–2%) and the animal positioned in the microPET P4 scanner (Tai et al., 2001). Image acquisition began approximately 60 min post-FDG administration and lasted for one hour. Anesthesia was maintained throughout the scan. Animals were maintained in metabolism cages for 24 hours after scanning, at which time radiation was decayed to background levels and animals were returned to their home cages.

PET images were reconstructed using a statistical algorithm resulting in images with an isotropic spatial resolution of approximately 1.8 mm (Qi et al., 1998). Because the size of the head is similar in all subjects, and comparisons are made across the same structure in different animals, the PET data were not corrected for photon attenuation or scatter. The reconstructed PET data are proportional to the metabolic rate for glucose (Phelps, 2000).

MRI Scanning

MRIs 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 hours before the procedure. At the start of the procedure, the male was sedated with Ketamine (10/mg/kg IM) and Medazolam (0.1mg/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 minutes 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 msec, repetition time TR = 22.0 msec, flip angle = 30.0 degrees, field of view = 8 cm, number of excitations = 3, matrix = 256 × 256, slice thickness = 1 mm. As a precautionary measure, the male's $EtCO_2$, oxygen saturation, heart rate and blood pressure were monitored throughout.

Image Registration

To spatially overlay the PET scans and the MRI scans we used RView (Division of Radiological Sciences, United Medical School of Guy's Hospital, London, United Kingdom). This image registration software package facilitates the image fusion of different modalities (e.g. MRI-PET, MRI-SPECT). The basic idea is to load multiple volume images into a single 3D “reference” space. In this case the anatomical MRI image was used as the “reference” volume and the PET volume was used as the “floating” image.

For each study the two data sets were loaded into RView and an approximate starting orientation for the PET data set was selected manually. The PET and MRI data sets were then registered with the RView auto-alignment algorithm, which works by maximizing the global mutual information between the two data sets (Studholme et al., 1997;Studholme et al., 1999). Where necessary a final manual alignment was performed based on structures clearly visible on both the PET and MRI images. After the alignment was completed the PET data set was transformed and resampled to the MR data set reference coordinates using trilinear interpolation.

RView was also used to manually segment the MRI data set into volumes of interest (VOIs). Each VOI was identified on the MRI slices and labeled in RView. Quantification of radiotracer uptake in these regions was performed using the previously generated transformed PET data set. The mean whole brain uptake was calculated by segmenting the brain for each study and quantifying the PET uptake in a similar manner.

Mean VOI values divided by whole-brain VOI value ("normalized units") were reported for each of the areas of interest. All VOI analysis was performed by a researcher blind to the assignment of animals to study groups and to the results of other tests performed, and confirmed by a second researcher. See Figure 1 for overlays of PET scans and MRIs, and Figures 2a and b for additional structural MRI images with neural regions indicated.

Cortisol Assay

Blood samples were collected on ice, centrifuged, and plasma stored at −80°C until time of assay. Time to sample collection averaged 275 seconds of removing the animal from the metabolism cage and did not differ significantly between treatment groups. Samples were assayed by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Intra-assay variation was 5.0%; inter-assay variation was 13.5%.

Data Analysis

In order to control for Type I error due to the large number of brain areas examined, we first analyzed differences between PB, LS, and LO males using a repeated measure multivariate analysis of variance (MANOVA) (Kleinbaum et al., 1988;O'Rourke et al., 2005) on two main groupings: 1) those areas implicated in pair-bonding by rodent studies, and 2) control areas implicated in locomotion (CP), fear (CeA), pain and parenting behavior (PAG), but not pair-bonding. Variables included the bonding status, hemisphere (as the repeated measure), and status by hemisphere interactions. A significant result from the MANOVA was followed by ANOVA on the individual neural areas in that grouping. Finally, post-hoc comparisons between social groups were carried out by least-squared means following significant ANOVA. All tests were two-tailed and significance was set at $p < 0.05$.

We also compared males before and after pairing by using paired t-tests. These tests were one-tailed as we predicted that changes in newly paired males would be in the direction of the long-term paired males.

Acknowledgments

This research was supported by the NIH base grant to the California National Primate Research Center (RR00169), a California National Primate Research Center pilot grant to K.L.B., NIH grant MH66756A to S.P.M., and a grant from the Good Nature Institute to K.L.B. We also gratefully acknowledge the following for research assistance: Richard Larson and the Center for Imaging Sciences at UC-Davis; Steve Rendig and Chris Griesemer of the Center for Molecular and Genomic Imaging; Carmel Stanko, Tessa Dutcher, and Vivian Imamura of the California National Primate Research Center; Drs. Cam Carter, Chris Machado, Ryan Walter and John Ryan, for advice on verifying the lesions; and Dr. Kari Christe for veterinary care.

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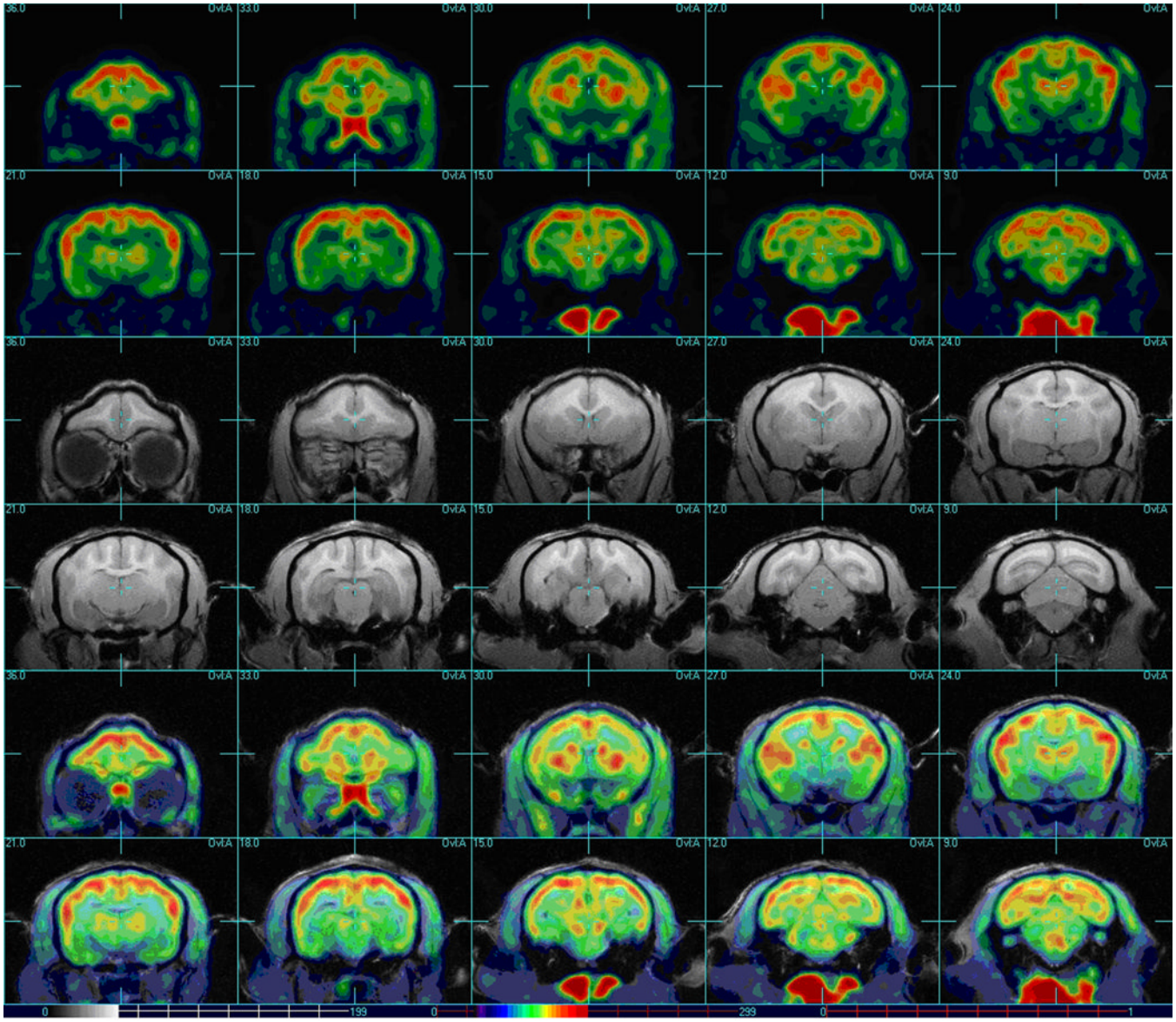

Figure 1.
Series of images rostral (from upper left) to caudal in the titi monkey brain. Rows 1 and 2 are PET images, Rows 3 and 4 are corresponding structural MRI images, and Rows 5 and 6 are the two sets of images overlaid.

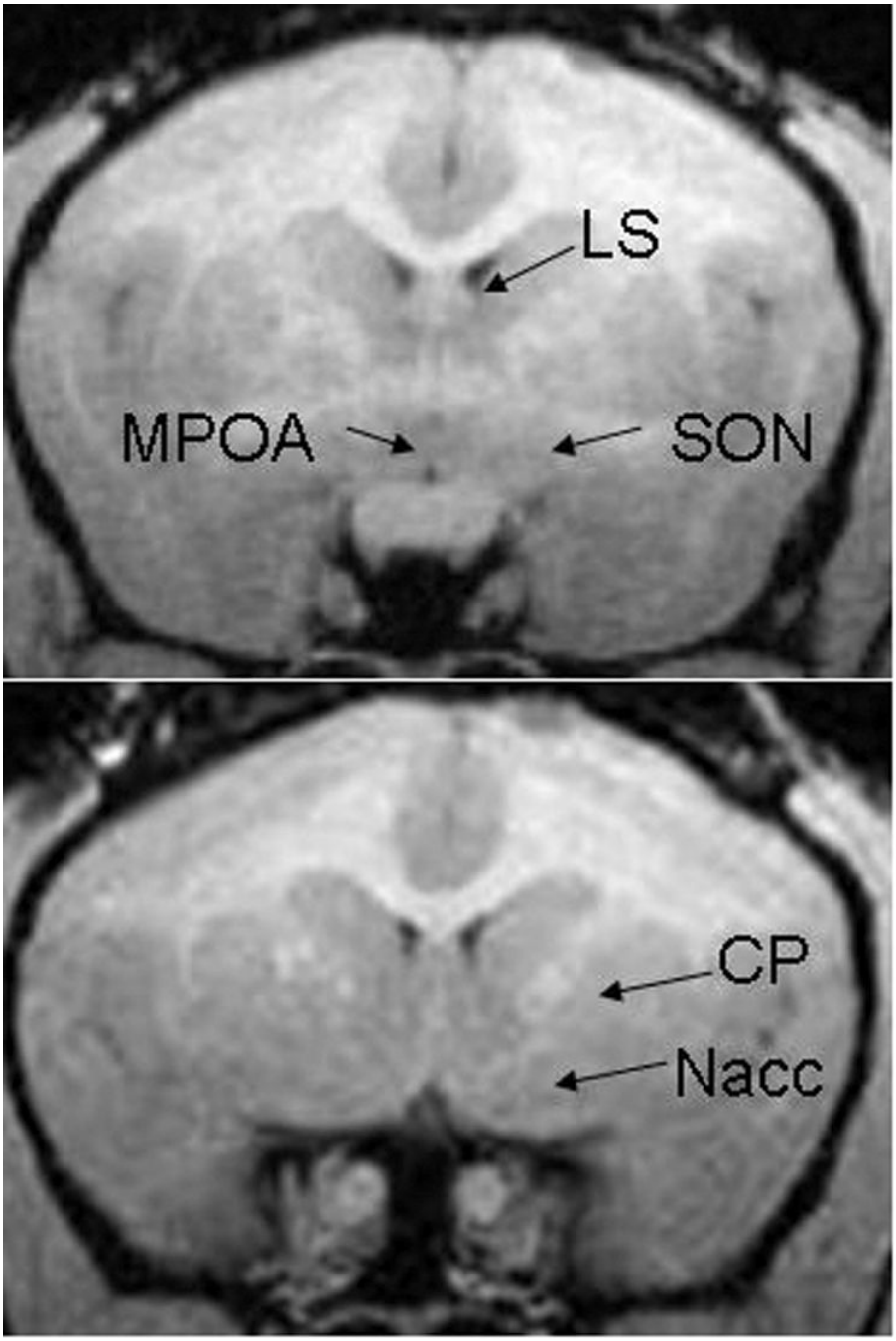

Figure 2.
Structural MRI images of a titi monkey brain at the level of (above) the anterior commissure and (below) the caudate-putamen. Nacc = nucleus accumbens, CP = caudate-putamen, LS = lateral septum, MPOA = medial preoptic area, SON = supraoptic nucleus.

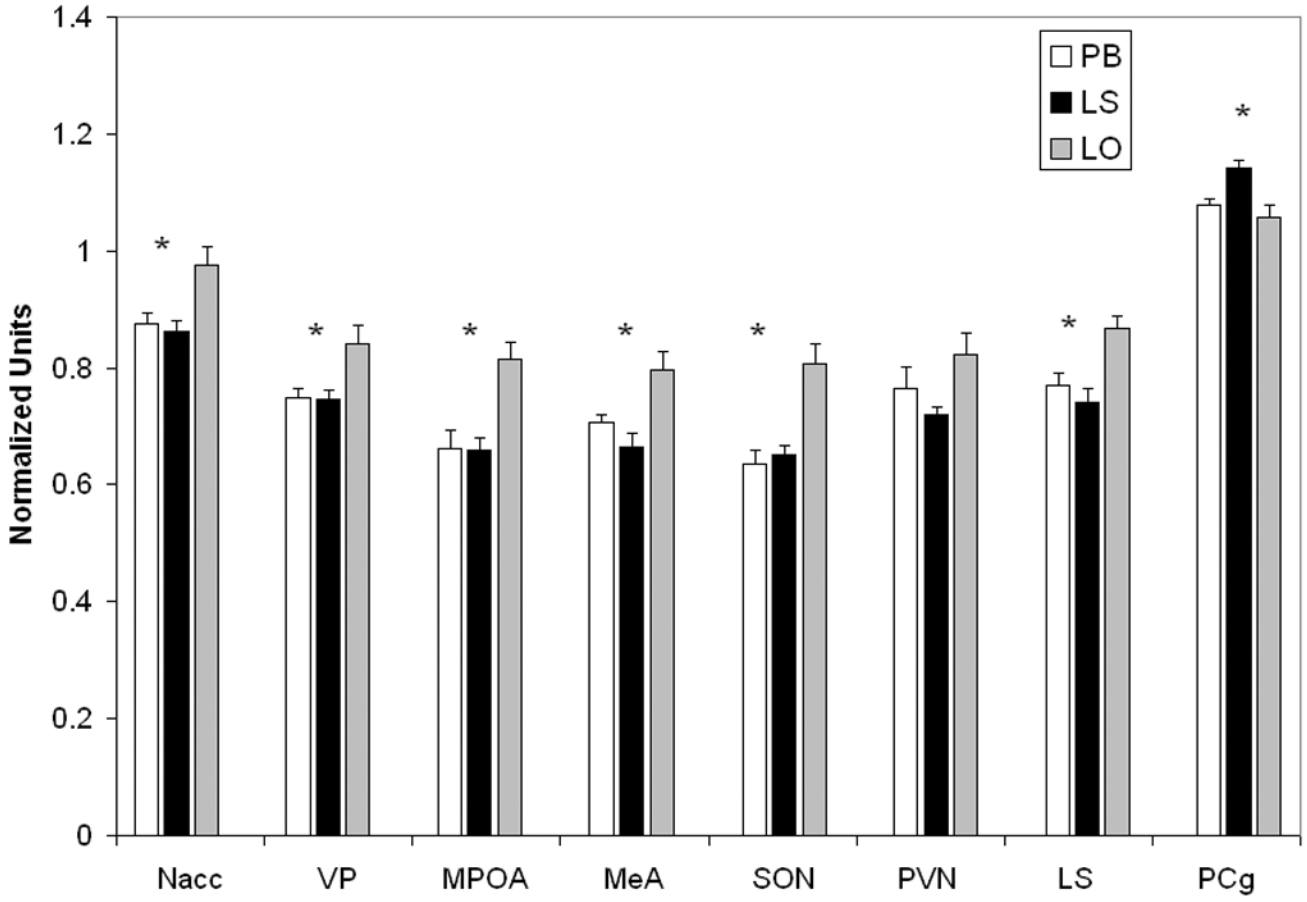

Figure 3.
Proportions of whole brain uptake per brain region, for regions predicted to be involved in pair-bonding. PB = pair-bonded (n = 6), LS = lesioned (n = 6), LO = lone (n = 5). * = $p < 0.05$. Neural regions = Nacc (nucleus accumbens), VP (ventral pallidum), MPOA (medial preoptic area), MeA (medial amygdala), SON (supraoptic nucleus of the hypothalamus), PVN (paraventricular nucleus of the hypothalamus), LS (lateral septum), and PCg (posterior cingulated cortex).

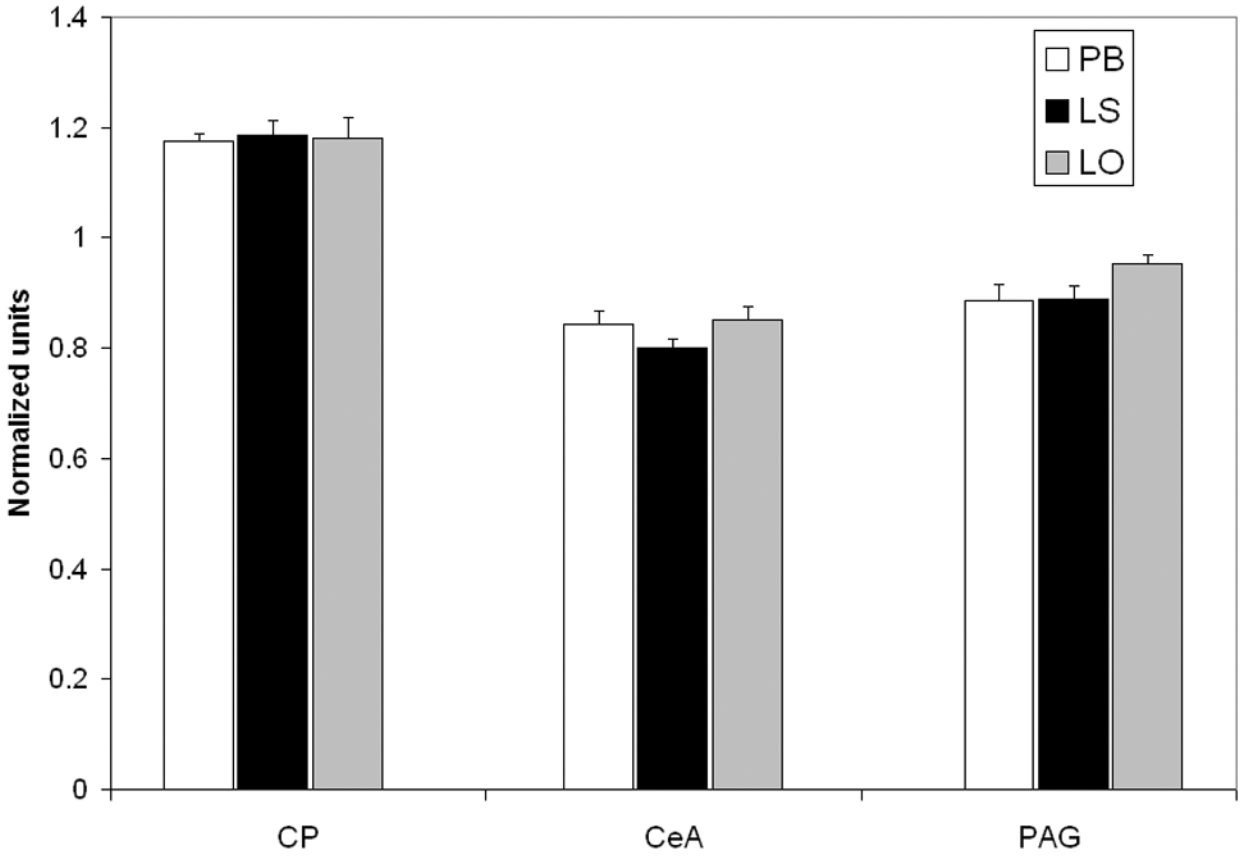

Figure 4.
Proportion of whole brain activity per neural area for control areas not predicted to be involved in pair-bonding. PB = pair-bonded (n = 6), LS = lesioned (n = 6), LO = lone (n = 5). Neural regions = CP (caudate-putamen), CeA (central nucleus of the amygdala), and PAG (periaqueductal gray of the midbrain).

Table 1

Subjects, birthdates, and pairing status. Five males were imaged both while lone and again while in newly formed pair-bonds.

Male ID	Male Birthdate	Pairing status
31716	04/27/99	Paired
29775	06/05/96	Paired
30060	03/31/97	Paired
30569	02/25/98	Paired
31768	05/13/99	Paired
29173	11/06/95	Paired
31267	10/21/98	Lesioned (paired)
30404	06/26/97	Lesioned (paired)
30070	04/02/97	Lesioned (paired)
29918	12/06/96	Lesioned (paired)
31289	02/02/99	Lesioned (paired)
30423	07/16/97	Lesioned (paired)
30410	07/02/97	Lone / Newly Paired
32878	03/06/01	Lone / Newly Paired
33442	06/08/01	Lone / Newly Paired
34531	01/10/03	Lone / Newly Paired
34387	08/13/02	Lone / Newly Paired

Table 2

Percent changes in glucose uptake in the right hemisphere for males before and 48 hours after pairing. Results of paired t-tests are shown (* = statistically significant result).

	Average Change (mean ± s.e.)	t-value	One-tailed p-value
Nacc	−2.5 ± 0.01%	2.86	0.023*
VP	−8.1 ± 3.5%	2.30	0.042*
MPOA	−6.8 ± 4.3%	1.61	0.091
MeA	−5.1 ± 2.7%	1.87	0.067
SON	−8.2 ± 3.9%	2.10	0.051
PVN	3.5 ± 5.7%	0.63	0.282
LS	4.7 ± 4.6%	1.00	0.186
PCg	−4.3 ± 4.1%	1.06	0.174