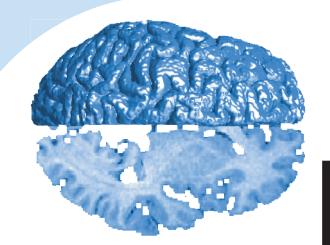


Patterns of Brain Activation Associated with Attachment Bond Disruption and Affiliative Vocalizations in Rhesus Monkeys: A FDG MicroPET Study

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Introduction

Extensive studies in rhesus monkeys have characterized the parameters of separation-induced vocalizations, or coo calls, as well as the neurochemical mechanisms that modulate their expression (Kalin & Shelton 1989).

Furthermore, it has been suggested that these high-pitched vocalizations are analogous to human cries (Newman, 1985)

Because of similarities between rhesus monkeys and humans in social behavior, and prefrontal cortex anatomy, rhesus monkeys are particularly well suited to study mechanisms underlying individual differences in response to separation (Kalin & Shelton, 2003).

In any given situation the degree to which an individual calls for help is related to not only the amount of distress that an individual experiences and the importance of social support at that time, but also the risks related to calling for help.

Research from our laboratory demonstrates that when separated monkeys are exposed to a potential threat, they decrease their calling for help (Kalin & Shelton, 1989).

In this study, we quantified the frequency of coo calls emitted by rhesus monkeys undergoing social separation and correlated individual differences in cooing with regional brain activity determined by [18F]-flouro-2-deoxy-D-glucose (FDG) small-animal high resolution PET.



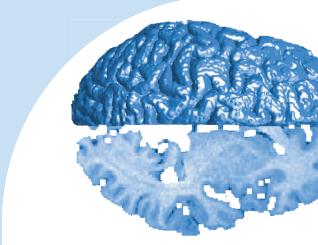
Methods

Subjects: Twenty-one male rhesus monkeys (*M. mulatta*) ranging in age from 2.2 to 4.6 years (mean age = 3.1 yrs.) and weighing between 3.2 and 7.4 kg (mean weight = 5.0 kg) were the subjects. The monkeys were pair housed and maintained on a 12-hour light/dark cycle at the Wisconsin National Primate Research Center and at the Harlow Primate Laboratory. Animals were given water ad libitum and were fed monkey chow every morning. Animal housing and experimental procedures were in accordance with institutional guidelines.

PET Acquisition: To minimize the nonspecific effects of handling, the animals were handled, given a mock injection and were placed in the test cage for 30 mins. on 5 different days. After this adaptation regime was completed, each animal was scanned on 2 separate occasions after being exposed to one of the three conditions of the modified human intruder paradigm (Kalin and Shelton, 1989). In this report, we present data from the ALN and NEC conditions. Scans were not performed more frequently than once per week and the order of the ALN and NEC was varied among the animals. The animals were food deprived overnight before the day of the PET scans. Between 8:00am and 1:40pm, the subjects were given a saphenous injection of approximately 7 milliCuries of the radiotracer FDG. Because greater than 70% of FDG uptake occurs within 30-40 minutes after injection (Rilling et al., 2001), the animals were immediately exposed to the paradigm and remained in experimental conditions for 30 mins. During ALN, the monkeys remained in the test cage for the entire 30-minute period. During NEC, a human entered the room for 10 minutes and presented her profile to the monkey, standing 2.5 meters from the cage and avoiding eye contact with the animal. To reduce the effects of habituation, the human left the test room for 5 minutes, reentered for 5 minutes, left again for 5 minutes, and reentered again for 5 minutes. Behaviors were recorded on videotape and were later rated with a computerized behavioral scoring system by trained raters unaware of the treatment conditions (Kalin and Shelton, 1989). Following the Human Intruder conditions the animals were anesthetized with ketamine (15 mg/kg) and were administered intramuscular atropine sulfate (0.27 mg) and were transported to the PET scanner facility. They were fitted with an endotracheal tube, to administer 1-2% isoflurane gas anesthesia. The subject's head was positioned in a stereotaxic apparatus, to maintain the exact same head position between conditions. The animal was then placed in the P4 microPET scanner (Concorde Microsystems, Inc., Knoxville, TN), a well characterized imaging system (Chatzioannou et al., 1999, 2000; Cherry et al., 1999; Knoess et al., 2003). The 60-minute emission scan was started on average 67 minutes (range, 58-83 mins) after injection of FDG. Heart rate, SpO₂, and respirations were monitored continuously. The microPET scanner has a reconstructed resolution of 2mm full width at half maximum (FWHM) yielding a volumetric resolution of approximately 8mm³.

Preprocessing: A crucial aspect of inter-subject comparisons is to obtain an accurate registration of the brains from all subjects into the same coordinate space. A multi-stage process with an MRI guided PET template was developed (Fox et al., Submitted, 2004) to obtain an acceptable level of accuracy within 2mm even for the subjects (n=15) in this study without MRI scans. An MRI template for those subjects with MRI scans (n=6) was created. PET scans for subjects with available MRI's were coregistered to match the original MRI. The deformations used to standardize the MRI images were then applied to the PET images. This method allowed for a clear distinction between brain and non-brain activations, as well as accurate localization of within-brain activation (Fox et al. Submitted, 2004). Each scan was globally normalized to a mean value, as blood sample collection would have interfered with the scanning environment. Since posterior brain areas were not included for all animals, areas more than 20mm posterior to the Anterior Commissure (AC) were not included in further analysis.

Quantification of Coo Calls: Coo vocalizations, defined as being made by rounding and pursing the lips with an increase and then a decrease in frequency and intensity, were recorded on videotape. Videotaped cooing was later rated with a computerized behavioral scoring system by trained raters unaware of the treatment conditions (Kalin and Shelton, 1989).



Results

A whole-brain voxelwise search, controlling for age, was performed to identify brain regions that were significantly correlated with individual differences in cooing (thresholded at p<.005, 2-tailed uncorrected).

The results revealed a positive correlation with activity in the right dorsolateral prefrontal cortex (dIPFC) (Area 46/9) and a negative correlation with activity in an area including dorsal right amygdala (Fig. 1a). Other brain regions significantly correlated with cooing during separation can be seen in Table 1. Since it is impossible to separate the contribution of muscle activity to brain activation in regions bordering metabolic muscles (Fox et al., Submitted, 2004), the region that included portions of the amygdala was intersected with a amygdala region of interest. This region was determined to be an acceptable distance from non-brain regions (Fox et al., Submitted, 2004), and was used for further analysis. No significant correlations were found between individual

differences in right dIPFC and dorsal right amygdala activity (p<.83). Furthermore, no significant correlations were found between the right dIPFC and locomotion (p<.99), and the right amygdala and locomotion (p<.37), confirming the specificity of the relation between metabolic activity in these areas and coo calls. The relation between dIPFC activity and cooing was lateralized as the correlation between cooing and dIPFC activity in the right hemisphere was significantly greater than that for the left hemisphere (p<.05, one-tailed). In contrast, the correlation between cooing and amygdala activity did not significantly differ between hemispheres (p<.08, one-tailed). A hierarchical regression was performed to understand the unique and overlapping contributions of the right dIPFC and amygdala to individual differences in cooing. Results demonstrated that activity in the right dIPFC and amygdala explained 76.3% of the variance (Fig. 1 c), with each area uniquely explaining approximately 33% of the variance (F(2,19)=30.623, p<.001) (Fig. 1 d).

Amygdala and Right dIPFC Predict Cooing

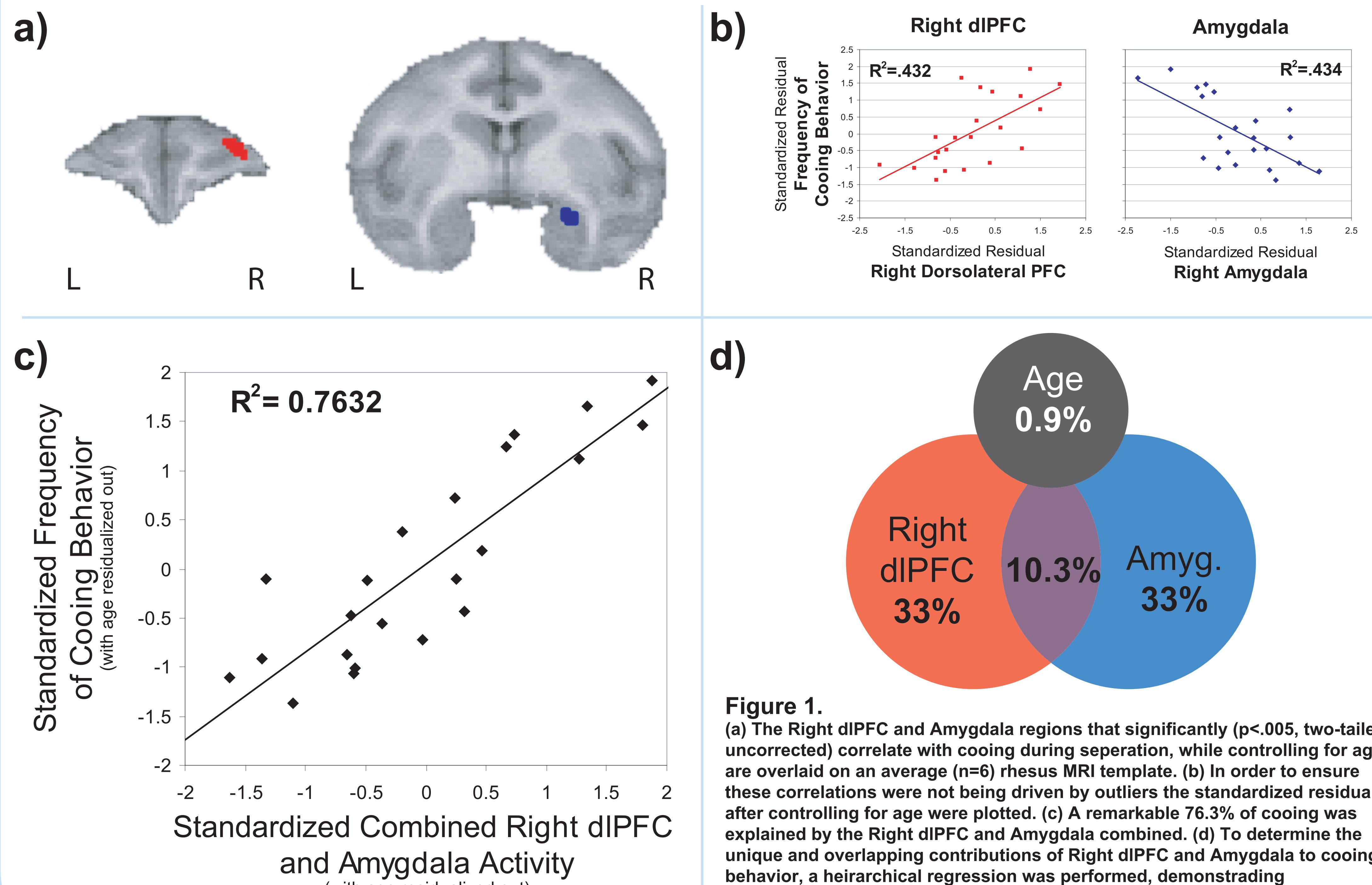


Figure 1.

(a) The Right dIPFC and Amygdala regions that significantly (p<.005, two-tailed uncorrected) correlate with cooing during separation, while controlling for age, are overlaid on an average (n=6) rhesus MRI template. (b) In order to ensure these correlations were not being driven by outliers the standardized residuals after controlling for age were plotted. (c) A remarkable 76.3% of cooing was explained by the Right dIPFC and Amygdala combined. (d) To determine the unique and overlapping contributions of Right dIPFC and Amygdala to cooing behavior, a hierarchical regression was performed, demonstrating approximately 33% of cooing behavior to be uniquely explained by each region.

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Table 1. Correlations With Cooing during ALN

			volume in mm ³	Max T-Value	Distance From AC/PC
Positive	PE/PEa (MIP)	Left	113	5.42	-11.875 -20 20
	PGM/Area 31	Left	20	3.58	-1.25 -21.875 13.125
Negative	dIPFC	Right	16	3.75	11.25 20.625 11.875
	Amygdala/Temporal Pole	Right	67	4.55	11.875 5 -6.875
	Lateral Geniculate	Right	48	4.48	14.375 -11.25 -3.125

Side Note: Context and Regulation

Since the adaptive response to the NEC condition is to decrease coo calls, we examined the relation between changes in brain activity between the ALN and NEC conditions with changes in the frequency of cooing across the conditions. Results demonstrated that individual differences in changes in brain activity in the right dIPFC were positively correlated (p<.005 two-tailed, uncorrected) with changes in cooing across the conditions (Figure 1). These findings suggest that the dIPFC is a region that is important in the expression of coo vocalizations.

Interestingly the correlation failed to include the same region of right dIPFC that was found to correlate with cooing behavior during the alone condition. In fact, the functional differences seem to coincide with the anatomical distinction between areas 46 and 46/9, which can be seen in figure 2 colored orange and green respectively. Since this also holds true at a decreased statistical threshold (p<.1), this suggests a unique role for area 46/9 in mediating cooing behavior during the alone condition.

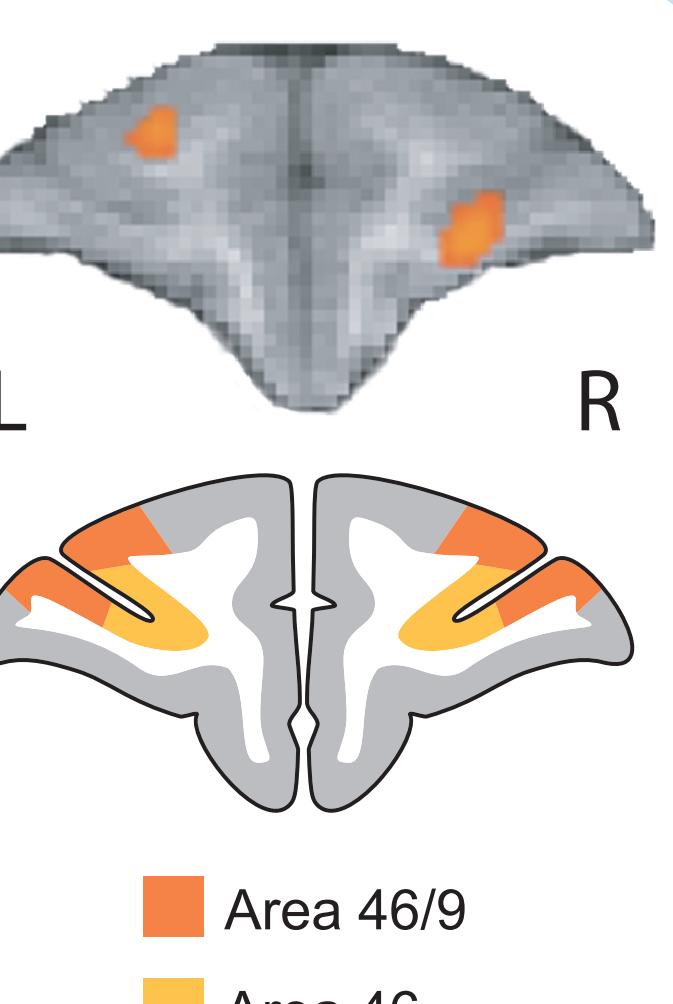


Figure 2

Discussion

Separation induces a state of loss and distress which motivates the production of calls for help.

The dIPFC has been proposed to act as an interface between emotion, cognition, and behaviour in maintaining emotional states that function to facilitate goal directed behaviour (Davidson & Irwin, 1999; Miller & Cohen, 2001; Gray et al. 2002; Wallis & Miller, 2003).

Other evidence from humans and monkeys demonstrates right asymmetric PFC activation in association with negative emotional states (Davidson & Fox, 1989; Davidson et al. 1992; Buss et al., 2003).

In addition, an FDG study in 6 young rhesus monkeys, demonstrated activation of the right dIPFC during maternal separation (Rilling et al., 2001).

The level of distress and associated need for reattachment increases coo calls, whereas they are decreased by the possibility of predatorial threat.

Since amygdala activity has been associated with threat detection, it is interesting to speculate that the negative relation between amygdala activity and cooing reflects an individual monkey's degree of perceived threat (Davis & Whalen, 2001).

This finding is consistent with recent studies in rhesus monkeys demonstrating that selective amygdala lesions increase cooing at the same time that they decrease fearfulness (Emery et al., 2001; Kalin et al., 2004; Bauman et al., 2004).

To summarize, we found that 76.3 % of the variance in the intensity of the separation response could be accounted for independent contributions of amygdala and dIPFC activity. This suggests that at least two separate neural networks, one reflecting distress and attachment and the other reflecting threat detection, are involved in modulating calls for help.