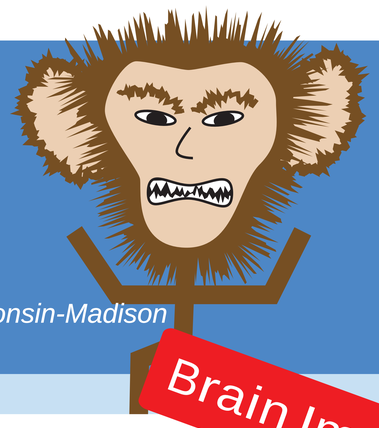
Threat-Related Modulation of Functional Relations Between Limbic and Prefrontal Regions.



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Introduction

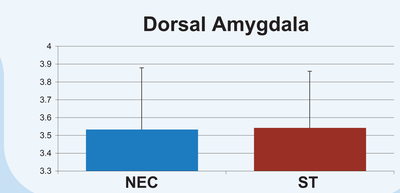
There is very little debate in the field of neuroscience that the amygdala is involved in emotional processing (Whalen 1998). Amygdalar involvement in vigilance and threat detection has been demonstrated across species (Davis & Whalen, 2001). Furthermore, functional coupling between prefrontal and amygdalar regions has been hypothesized to underlie the regulation of emotions and behaviors associated with adaptive responding .

In this study we investigated how brain activity in the dorsal amygdala is related to prefrontal regions under different threatening conditions in freely moving, unperturbed monkeys.

Threat was manipulated by altering the actions of a human intruder. The experimenter entered the testing room, and presented the monkey with either No Eye Contact (NEC) or her direct gaze (Stare, ST) (Kalin & Shelton, 1989).

All correlations were done across subjects, investigating the relation between integrated glucose metabolism over a 30-minute time period in the dorsal amygdala and its relation to prefrontal areas.

Observations that the amygdala habituates quickly (Zald, 2002), suggest that over the course of ~30-minute uptake period we would not observe significant differences in amygdala activation. This was true, there was no significant difference between dorsal amygdala glucose metabolism between conditions.



Methods

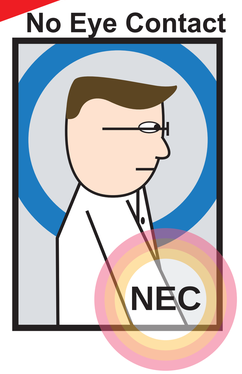
Subjects:
Twenty-one male rhesus monkeys (M. Mulatta) ranging in age from 2.2 to 4.6 years (mean age = 3.1 years) and weighing between 3.2 and 7.4 kg (mean weigh = 5.0 kg) were the subjects. The monkeys were pair housed and maintained on a 12-hour light/dark cycle at the Wisconsin National Primate Research Center 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.

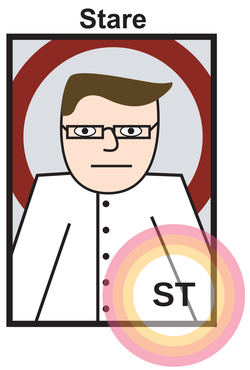
PET Acquisition:
To minimize the nonspecific effects of handling, the animals were handled, given a mock injection and were placed in the test cage for 30 minutes on 5 different days. After this adaptation regime was completed, each animal was scanned on 2 separate occasions after being exposed to one of the three conditions of the modified human intruder paradigm (Kalin & Shelton, 1989). In this report, we present data from the NEC and ST conditions. Scans were not performed more frequently than once per week and the order of the NEC and ST conditions was varied among the animals. The animals were food deprived overnight before the day of PET scans. Between 8:00am and 1:40pm, the subjects were given a saphenous injection of approximately 5 milliCuries of the radiotracer [18F]-flouro-2-deoxy-D-glucose (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 minutes. 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, and reentered again for 5 minutes. The ST condition was identical, with the exception that the human stared at the monkey when she was in the room. Behaviors were recorded on videotape and were later rated with a computerized behavioral scorind system by trained raters unaware of the treatment conditions (Kalin & Shelton 1989). Following the Human Intruder conditions, the animals were anesthetized with ketamine (15 mg/kg) and were administered intramuscular atropine sulfate (0.27mg) and were transported to the PET facility. They were fitted with an enotracheal tube, to administer 1-2% isofluorane gas anesthesia. The subjects 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 (Chatziioannou et al., 1999, 2000; Cherry et al., 1997; Farquhar et al., 1998; Knoess et al., 2003). The 60-minute emission scan was started on average 67 minutes (range = 58-83 minutes) 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.

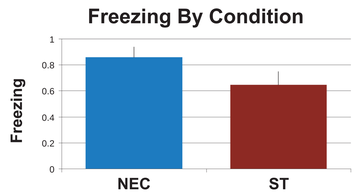
Preprocessing:
A crucial aspect of inter-subject comparisons is to obtain an accurate registration of the brains from all subjects into the same coordinate space. A multi-stage process with an MRI guided PET template was developed (Kalin et al., 2005; Fox et al., In Prep) to obtain an acceptable level 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 images. The deformations used to standardize the MRI images were then applied to the PET imagines. 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., In Prep). 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 no included for all animals, areas more than 20mm posterior to the anterior commisure (AC) were not included in further analyses.

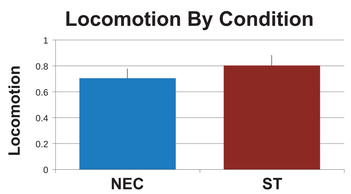
Statistical Analyses:
Dorsal amygdala activity was assessed by extracting the mean glucose metabolism within a region of interest around the dorsal amygdala (defined by SES & NHK). We then performed a voxelwise search throughout the prefrontal cortex for regions that correlated with dorsal amygdala activation, controlling for age, in both NEC and ST conditions. A voxelwise search was then performed on the resulting statistical maps, searching for areas of the prefrontal cortex that significantly differed in their relationship with the amygdala between the NEC and ST conditions.

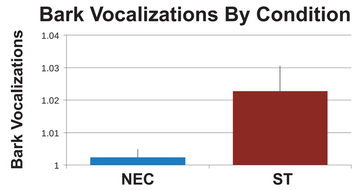
Behavioral

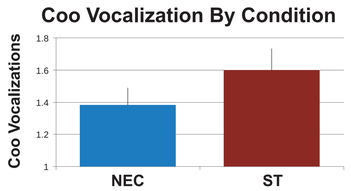






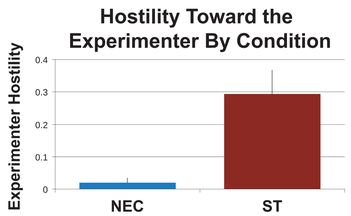






Behavior Definitions

Locomotion: Ambulation of one or more full steps at any speed. Includes such behaviors as dropping from ceiling to floor or swinging cage shake. May be scored with other behaviors. Scored as the log of the duration in seconds plus one.
Freezing: A period of at least three seconds characterized by tense body posture, no vocalizations and no movement other than slow movements of the head. Scored as the log of the duration in seconds plus one.
Experimenter Hostility: Any hostile behaviors directed at the tester, e.g. barking, head bobbing, ear flapping etc. Scored as the log of the duration in seconds plus one.
Coo: Vocalization made by rounding and pursing the lips with an increase then decrease in frequency and intensity. Scored as the square root of frequency per minute plus one.
Bark: Vocalization made by forcing air through vocal chords from the abdomen producing a short, rasping low frequency sound. Scored as the square root of frequency per minute plus one.



Results Summary

Behaviors:
During NEC, the monkeys engaged in significantly more freezing behavior. In contrast, during ST monkeys demonstrated significantly more hostility toward the experimenter, locomotion, and vocalizations.

Brain Activity:
During NEC, activity in the dorsal amygdala was positively correlated with activity in the anterior cingulate (areas 24c and 32/9) and dorsolateral (areas 46V and 46/9V) prefrontal regions (r=.468, p=.037). During ST this relationship was reversed (r=-.582, p=.007). These correlations are significantly different (p<.05). There was no significant difference between activity in the amygdala or prefrontal cluster between conditions. There were no significant correlations between amygdala or prefrontal clusters and observed behaviors (all p's>.05).

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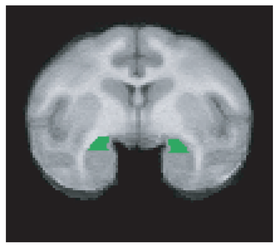
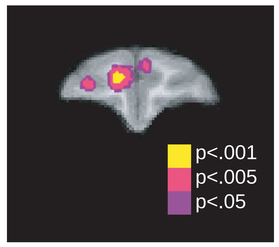
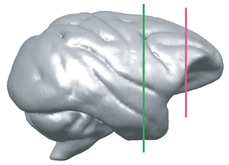
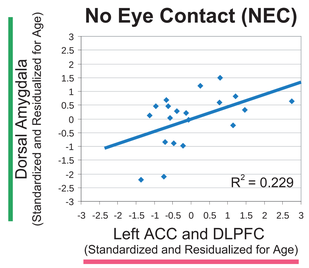
Brain Imaging

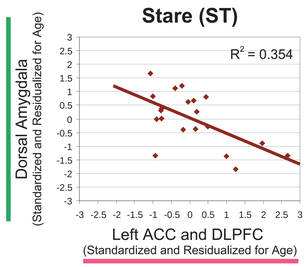
Correlations between Dorsal Amygdala and PFC differ between the NEC and ST conditions











Discussion

These findings are interesting because the adaptive responses to NEC involve behavioral inhibition and constant risk assessment, which is thought to involve anterior cingulate. During ST the adaptive response is no longer to risk assess, but to actively engage in protective behaviors. Furthermore, the negative relation between anterior cingulate and dorsal amygdala may reflect inhibitory influences of PFC on amygdala that underlies the shift from risk assessment to action and emotion regulation.

Though the kinetics of FDG did not allow us to investigate the timecourse of amygdalar-prefrontal dynamics, research in humans has demonstrated contextual modulation of amygdala activity to be functionally related to PFC function using fMRI in both normal subjects (Kim et al., 2004) and phobic patients (Lorberbaum et al., 2004). In monkeys, anatomical studies have demonstrated strong connections between the anterior cingulate and amygdalar regions (Stefanacci & Amaral, 2002; Croxson et al., 2005). Furthermore, this region has been implicated in extinction learning, which may utilize the same core components as emotion regulation. We believe this data, taken together, implicates a prefrontal-amygdalar circuit in successfully contextually regulating behaviors.

Future work should attempt to further asses behavioral relationships that relate to this brain network in order to appropriately identify it's significance.