Brain Regions Associated with the Expression and Contextual Regulation of Anxiety in Primates

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Background: *A key to successful adaptation is the ability to regulate emotional responses in relation to changing environmental demands or contexts.*

Methods: *High-resolution PET ^{18}fluoro-deoxyglucose (FDG) scanning in rhesus monkeys was performed during two contexts (alone, and human intruder with no eye contact) during which the duration of anxiety related freezing behavior was assessed. Correlations between individual differences in freezing duration and brain activity were performed for each of the two conditions, as well as for the contextual regulation between the two conditions.*

Results: *In both conditions, activity in the basal forebrain, including the bed nucleus of the stria terminalis and the nucleus accumbens were correlated with individual differences in freezing duration. In contrast, individual differences in the ability to regulate freezing behavior between contexts were correlated with activity in the dorsal anterior cingulate cortex, the thalamus and the dorsal raphe nucleus.*

Conclusions: *These findings demonstrate differences in the neural circuitry mediating the expression compared to the contextual regulation of freezing behavior. These findings are relevant since altered regulatory processes may underlie anxiety disorders.*

Key Words: Anxiety, freezing, PET, monkey, emotion, regulation

The adaptive expression of emotion relates to the ability to regulate emotional responses as environmental or contextual demands change (Kalin and Shelton 1989; Davidson et al 2000; Kalin and Shelton 2003). Furthermore, it is likely that there are differences in the neural circuitry underlying the regulation, compared to the expression of emotion (Davidson et al 2000; Kalin and Shelton 2003; Morgan and LeDoux 1999; Ochsner et al 2002). Previously, we demonstrated that rhesus monkeys provide an excellent model to study mechanisms underlying human anxiety, its regulation, and anxiety-related psychopathology (Kalin 1993).

When threatened, primates commonly engage in behavioral inhibition or freezing behavior. Freezing is an automatic response characterized by the cessation of motor and vocal activity. In monkeys, individual differences in freezing duration are stable over time and reflect individual levels of anxiety (Kalin and Shelton 1989). Adaptive freezing helps an individual remain inconspicuous and decreases the likelihood of predatorial attack; however, excessive freezing, or behavioral inhibition, is a risk factor for the development of anxiety disorders (Kagan et al 1998; Biederman et al 2001).

The human intruder paradigm was developed to study primates' defensive behaviors, such as freezing, and their regulation in response to changing environmental demands (Kalin and Shelton 1989). In this regard, it is informative to compare monkeys' responses elicited by attachment bond disruption occurring during the alone condition (ALN) with the responses elicited by threat occurring during the no eye contact condition (NEC). During ALN, monkeys are separated from their cagemates into a novel environment. Initially, monkeys display some freezing which functions to decrease the chances of detection by a potential predator. Once the monkey establishes that in the ALN environment there is no real predatorial threat, it locomotes and emits coo calls. These behaviors increase the likelihood of reunion by attracting the attention of conspecifics. Compared to ALN, the NEC condition provides a very different context introducing a direct threat. During NEC, the monkeys are exposed to a human intruder that presents her profile to the monkeys while avoiding eye contact. The lack of eye contact increases the uncertainty of the potential predator's intent and as a result the monkey engages in longer durations of freezing. This increased freezing compared to that during the ALN condition reflects the monkey's ability to adaptively regulate its anxiety-related behavior.

To understand the neural circuitry associated with freezing and its contextual regulation, ^{18}fluoro-deoxyglucose (FDG) PET scans were performed in monkeys exposed to the ALN and NEC conditions on separate days. FDG was selected as the radiotracer because its uptake into brain regions reflects brain metabolic activity (Sokoloff et al 1977; Phelps et al 1979). FDG was administered immediately before placing the monkeys in ALN or NEC conditions in which they remained for 30 min.

Based on previous work, we expected that individual differences in freezing duration would be correlated with activity in the amygdala, bed nucleus of the stria terminalis (BNST), and the periaqueductal gray (Davis et al 1997; LeDoux et al 1988; Davis 2000; Kalin et al 2004). To assess brain regions involved in the regulation of freezing, the change in freezing behavior between ALN and NEC was measured for each animal, and was correlated with changes in brain activity between these conditions. We hypothesized that individual differences in the regulation of freezing would be associated with the activation of regions of the prefrontal and anterior cingulate cortex (Ochsner et al 2002).

Methods and Materials

Subjects

Twenty-five 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 weight = 5.0 kg) were the subjects. The monkeys were pair housed and maintained on a

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12-hour light/dark cycle at the Wisconsin National Primate Research Center and at the Harlow Primate Laboratory. Animals had water ad libitum and were fed monkey chow every morning. Animal housing and experimental procedures were in accordance with institutional guidelines.

Magnetic Resonance Imaging Methods

Magnetic resonance imaging (MRI) data were available from 6 of the monkeys. Data were collected using a GE Signa 3T scanner (GE Medical Systems, Milwaukee, Wisconsin) with a standard quadrature birdcage headcoil. Whole brain anatomical images were acquired using an axial 3D T1-weighted inversion-recovery fast gradient echo sequence (TR = 9.4 msec, TE 2.1 msec, FOV = 14 cm, flip angle = 10°, NEX = 2, matrix = 512 × 512, voxel size = .3 mm, 248 slices, slice thickness = 1 mm, slice gap = −.05 mm, prep time = 600, bandwidth = 15.63, frequency = 256, phase = 224). Before undergoing MRI acquisition, the monkeys were anesthetized with intramuscular ketamine (15 mg/kg).

PET Scan Protocol

To minimize the nonspecific effects of handling, the animals were handled, restrained and given a mock injection, and placed in the test cage for 30 min on 5 different days. After adaptation, each animal was scanned on 3 separate occasions after exposure to one of the conditions of the modified human intruder paradigm (Kalin and Shelton 1989). In this report, we present data from the ALN and NEC conditions. Data from the stare condition is not presented at this time because of our interest in understanding the regulation of freezing between the ALN and NEC conditions. Scans were not performed more frequently than once per week with 14 animals exposed to ALN first and 11 animals exposed to NEC first. The animals were food deprived overnight and between 8:00 am and 1:40 pm, the subjects were injected with <10 milliCuries (mCi) of the radiotracer FDG through a 19 ga intravenous (IV) catheter in the saphenous vein. Because greater than 70% of FDG uptake occurs within 30-40 min after injection (see e.g. Rilling et al 2001), the animals were immediately exposed to the paradigm and remained in the experimental conditions for 30 min. After each experimental session, the monkeys were anesthetized with ketamine followed by isofluorane gas, and their local glucose metabolism was assessed. Though previous studies demonstrate that ketamine (Langsjo et al 2003, 2004; Holcomb et al 2001; Freo and Ori 2004; Honey et al 2004) and isofluorane (Alkire et al 1997) produce changes in metabolism or rCBF in a variety of species (rats monkeys, humans), they all demonstrate that the response within a brain region is relatively constant across subjects. It is important to note that similar experimental timing was used for all subjects and that the anesthesia was identical in both conditions. During ALN, the monkeys remained alone in the test cage for the entire 30-min period. During NEC, the monkeys were place in the same cage for the entire 30-min period while a human entered the room for 10 min 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 min, reentered for 5 min, left again for 5 min, and reentered again for 5 min. Behaviors were recorded on videotape and were rated with a computerized scoring system by trained raters unaware of the treatment conditions (Kalin and Shelton 1989). Freezing was defined as a complete cessation of movement with the exception of vigilant eye movements that lasted for a minimum of three sec (Kalin and Shelton 1989). Following the Human Intruder conditions the animals were anesthetized with ketamine (15 mg/kg) and were administered intramuscular atropine sulfate (.27 mg). They were then fitted with an endotracheal tube, to administer 1–2% isofluorane 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, Tennessee), a well characterized imaging system (Chatziioannou et al 1999, 2000; Cherry et al 1997; Farquhar et al 1998; Knoess et al 2003). The 60-min emission scan was started on average 67 min (range, 58–83 min) after injection of FDG. Heart rate, SpO_2, and respirations were monitored continuously. The delay between the activation paradigm and the time to scan, does not affect the relative metabolic activity among brain regions since with single bolus injections, FDG decays at the same rate in all parts of the brain. The microPET scanner has a reconstructed resolution of 2 mm full width at half maximum (FWHM) yielding a volumetric resolution of approximately 8 mm^3.

Post Acquisition Processing

To compare data across subjects, each PET scan was transformed into the same coordinate system using standard analysis methodology. A crucial aspect of whole brain inter-subject comparisons is to obtain an accurate fit of the brains from all subjects into the same coordinate space, such that each brain structure resides in the same location for all subjects. A multistage process was used to create study-specific MRI and PET templates and accurately align each PET scan to the PET template. To facilitate the creation of an MRI template, each MRI scan was segmented into brain and nonbrain tissue, so that differences in skull and muscular anatomy would not influence the fit of the brain. One MRI image was manually transformed into a standard "template space" as defined by Paxinos et al (2000). The remaining MRIs were transformed linearly to match the one standardized image with a shift, a rotation, and a zoom transformation in each of the three (x, y, z) dimensions using AIR (9-parameter model) (Woods et al 1998). For each of the six subjects with MRI scans, reconstructed FDG images were transformed linearly via a shift and a rotation transformation in each of three-dimensions (x, y, z) to match their original MRIs (6-parameter rigid-body model). The 9-parameter transformations attained from the MRI transformations were then applied to the PET data, which were averaged to create a PET template in standardized space. This template was then masked to include the brain and surrounding muscles that bordered the brain, yielding a BRAIN-PET template. All subjects exhibited a similar pattern of extra-brain activity.

PET images from each subject were then matched to the template image. First, reconstructed PET images from each subject were smoothed with a 4 mm FWHM Gaussian kernel to accommodate small differences in the locations of functional regions across subjects (e.g. due to differences in gyral features) and increase the signal to noise ratio (Worsley et al 1996). Each PET image was then transformed into template space using a shift, a rotation, a zoom, and a perspective transformation in each of the three dimensions (x, y, and z) to match the location of the brain to the unmasked PET template using FSL (12-parameter affine model) (Jenkinson et al 2002). This transformation matched the size, location and orientation of each brain to that of the PET template. Each PET image was masked to exclude obvious nonbrain areas using the same mask applied to the BRAIN-PET template. Since posterior brain areas were not acquired for some scans due to the small field of view in the PET