Activity and Connectivity of Brain Mood Regulating Circuit in Depression: A Functional Magnetic Resonance Study

Amit Anand, Yu Li, Yang Wang, Jingwei Wu, Sujuan Gao, Lubna Bukhari, Vincent P. Mathews, Andrew Kalnin, and Mark J. Lowe

Background: *Functional imaging studies indicate that imbalances in cortico-limbic activity and connectivity may underlie the pathophysiology of MDD. In this study, using functional Magnetic Resonance Imaging (fMRI), we investigated differences in cortico-limbic activity and connectivity between depressed patients and healthy controls.*

Methods: *Fifteen unmedicated unipolar depressed patients and 15 matched healthy subjects underwent fMRI during which they first completed a conventional block-design activation experiment in which they were exposed to negative and neutral pictures. Next, low frequency blood oxygenation dependent (BOLD) related fluctuations (LFBF) data were acquired at rest and during steady-state exposure to neutral, positive and negative pictures. LFBF correlations were calculated between anterior cingulate cortex (ACC) and limbic regions – amygdala (AMYG), pallidostriatum (PST) and medial thalamus (MTHAL) and used as a measure of cortico-limbic connectivity.*

Results: *Depressed patients had increased activation of cortical and limbic regions. At rest and during exposure to neutral, positive, and negative pictures cortico-limbic LFBF correlations were decreased in depressed patients compared to healthy subjects.*

Conclusions: *The finding of increased activation of limbic regions and decreased LFBF correlations between ACC and limbic regions is consistent with the hypothesis that decreased cortical regulation of limbic activation in response to negative stimuli may be present in depression.*

Key Words: Depression, fMRI, cortico-limbic, connectivity, emotional valence, mood circuit, low frequency BOLD fluctuations

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have reported decreased activation of cortical regions such as the dorsolateral prefrontal cortex (DLPFC) (Ketter 1996; Mayberg et al 1999) and anterior cingulate cortex (ACC) (Drevets et al 1997) in depression. Conversely, increased activation of limbic regions such as medial thalamus (MTHAL), pallidostriatum (PST) and amygdala (AMYG) (Drevets 2000b; Mayberg et al 1999; Sheline et al 2001; Siegle et al 2002) has been reported in depression. This pattern of regional activation in depression has led to postulation of a putative prefrontal-amygdalar-pallidostriatal-mediothalamic mood regulating circuit (MRC) (Anand and Charney 1999; Drevets 1998; Mayberg 1997). Depression has been postulated to arise due to imbalances in connectivity in this circuit leading to decreased regulatory effect of the cortical areas such as the ACC over the limbic regions such as AMYG, PST and MTHAL (Damasio 1997; Drevets et al 1997; Mayberg et al 1999). Recent advances in brain imaging and image analysis have made it possible to study connectivity between brain regions in humans, in vivo.

Functional connectivity between brain regions has been defined as the temporal correlation between spatially remote neurophysiological events (Friston et al 1993). Current research indicates that the production of emotions may be dependent on a distributed neuronal network consisting of cortical and limbic regions rather than on the activity of a discrete brain region (Damasio 1997). Therefore, brain abnormalities in mood disorders are much more likely to be present in functional connectivity between brain regions, rather than within discrete brain regions (Mayberg 2003).

Brain imaging paradigms which have sought to explore functional connectivity (Friston et al 1993) have done so by correlating activation of brain regions working on the assumption that if the activation of two brain regions in response to a task is correlated, then they are likely to be functionally connected (Lawrie et al 2002; McIntosh et al 1994; Menon et al 2001; Meyer-Lindenberg et al 2001; Stephan et al 2001). A number of investigators have reported promising results using this method. However, in this paradigm, there is a possibility that similar activation may be seen in areas with different resting states and different levels of BOLD changes (Shulman 2001). Moreover, functionally unconnected regions may respond similarly to changes associated with a task. Recent studies, which have used PET to measure resting state blood glucose metabolism, have reported changes in cortico-limbic circuitry after treatment in depressed patients (Mayberg 2002; Seminowicz et al 2004). However, until recently, methods were not available to measure steady-state blood flow changes using fMRI.

In a recently developed method, correlation of low frequency BOLD- weighted temporal fluctuations (LFBF) in steady-state fMRI data has been used as a measure of connectivity between brain regions (Biswal et al 1995; Lowe et al 2000). Spontaneous low-frequency oscillations in regional cerebral blood flow and oxygenation in animals have been observed with laser Doppler flow, fluororeflectometry, fluorescence video microscopy, and polarographic measurement of brain tissue (Lowe et al 2002). Biswal and colleagues demonstrated that very low frequency ($< .08$ Hz) temporal fluctuations in BOLD weighted echoplanar imaging data are phase locked between areas of plausible functional connectivity (Biswal et al 1995). It has recently been

From the Department of Psychiatry (AA, YL, LB), Department of Radiology (AA, YW, VPM, AK), and Department of Medicine (JW, SG), Indiana University School of Medicine, Indianapolis, Indiana; Division of Radiology (MJL), The Cleveland Clinic Foundation, Cleveland, Ohio.

Address reprint requests to Amit Anand, M.D., Outpatient Psychiatry Clinic, University Hospital Suite #3124, 550 N. University Boulevard, Indianapolis, IN 46202; E-mail: aanand@iupui.edu.

Received August 12, 2004; revised January 24, 2005; accepted February 14, 2005.

0006-3223/05/$30.00
doi:10.1016/j.biopsych.2005.02.021

recognized that these LFBFs (< .08 Hz) are not associated with features of any task or overt stimulus, and are not caused by instrumentation or physiological effects (such as cardiac and respiratory cycles) originating outside the brain (Biswal et al 1995) and that these resting state signal changes reflect alterations in blood flow and oxygenation that may be coupled to neuronal activity (Maldjian 2001). Correlation of LFBF as a measure of functional connectivity between distant functionally related brain regions has been used successfully to demonstrate connectivity between brain regions which are known to be functionally related from neurophysiological and neurological studies – e.g. DLPFC and middle frontal gyrus during performance of a finger tapping task (Lowe et al 2000), sensorimotor and language areas (Arfanakis et al 2000), Broca's and Wernicke's area while performance of a speech task (Hampson et al 2002), and cingulate cortex and DLPFC during performance of a working memory task (Greicius et al 2003). Therefore, using fMRI, LFBF correlations may be useful for measuring cortico-limbic connectivity.

In this study, we tested the hypothesis using conventional block-design task (emotionally valenced pictures)-generated fMRI, whether depressed patients have abnormal activation of the mood regulating areas of the brain. We further hypothesized that decreased connectivity between the ACC and limbic regions such as AMYG, PST and MTHAL as measured by decreased phase coherence between LFBFs measured in these areas may be present in depressed patients compared to healthy controls. We tested the hypothesis, using resting state LFBF correlations, whether decreased cortico-limbic connectivity was present in depressed patients compared to healthy subjects. However, differences in cortico-limbic connectivity between patients and healthy controls may become more apparent while performing a task that recruits the functioning of the cortico-limbic circuit (e.g., exposure to an emotionally valenced stimulus). Therefore we also investigated LFBF correlations differences between depressed patients and healthy controls on exposure to neutral, positive and negative pictures.

Methods and Materials

Subjects

Medication free unipolar depressed outpatients were recruited from the outpatient clinic at University Hospital, Indiana University School of Medicine and by advertisement. Healthy subjects matched for age, sex and ethnicity were also recruited via advertisement. All subjects took part after signing an informed consent form approved by the Investigational Review Board (IRB) at Indiana University School of Medicine. Both patients and subjects were paid $50 for screening and $50 for MRI scan. Inclusion criteria for depressed subjects were: age 18–60 years and able to give voluntary informed consent; satisfy Diagnostic and Statistical Manual fourth edition (DSM-IV) criteria for Major Depressive Episode; 25-item Hamilton Depression Rating Scale (HDRS) score > 18; satisfy criteria to undergo an MRI scan based on MRI screening questionnaire; and be able to be managed as outpatients. Exclusion criteria for depressed patients were: meeting DSM-IV criteria for schizophrenia, schizoaffective disorder, bipolar disorder or an anxiety disorder as a primary diagnosis; use of psychotropics in the past 2 weeks; use of fluoxetine in the past 4 weeks; acutely suicidal or homicidal or requiring inpatient treatment; meeting DSM-IV criteria for substance dependence within the past year, except caffeine or nicotine; positive urinary toxicology screening at baseline; use of alcohol in the past week; serious medical or neurological illness; current pregnancy or breastfeeding; and metallic implants or other contraindications to MRI. Inclusion criteria for healthy subjects were: ages 18–60 years and able to give voluntary informed consent; no history of psychiatric illness or substance abuse or dependence; no family history of major psychiatric or neurological illness in first degree relatives; not currently taking any prescription or centrally acting medications; no use of alcohol in the past week; and no serious medical or neurological illness. Exclusion criteria for healthy subjects were: under 18 years of age; pregnant or breastfeeding; and metallic implants or other contraindication to MRI.

After complete description of the study to the subjects, written informed consent was obtained.

Behavioral Ratings

Subjects were rated on 25-item Hamilton Depression Rating Scale (HDRS) (Thase et al 1991) at screening and on day of scan.

Visual Picture Sequence. The International Affective Picture System (IAPS) (Lang, Bradley and Cuthbert, National Institute of Mental Health (NIMH) Center for the Study of Emotion and Attention) (Lang et al 1997) is a large set of standardized, emotionally-evocative, internationally accessible color photographs. The IAPS pictures are rated on two primary dimensions – affective valence and arousal. Each picture is rated on a 9 point scale such that 9 represents a high rating on each dimension (high pleasure, high arousal) and 1 represents a low rating (low pleasure, low arousal). For the purpose of this study, we decided to include, ratings applicable to sexes, negative pictures (valence ratings 2 – 3; arousal ratings < 6), positive pictures (valence ratings > 7.5 and arousal ratings < 6), and neutral pictures (valence ratings 4.5 – 5.5 and arousal < 3). Different picture sets were given for the fMRI activation scan and the LFBF component of the experiment.

Functional MRI Scan

Image Acquisition. Image data were acquired using a General Electric (Waukesha, Wisconsin) 1.5 T MRI scanner. Subjects were placed in a birdcage head coil and individually fitted to a bite bar partially composed of dental impression compound attached to the coil to reduce head motion. Visual stimuli for all tasks were computer generated and presented using an MRI compatible binocular fiberoptic goggles (Avotec Inc., Jensen Beach, Florida). Before the scan, subjects were instructed to just look at the pictures and let the feelings elicited by the pictures flow and not try to suppress the feelings elicited by the pictures. Subjects were not asked to rate the pictures or perform any other cognitive task, as cognitive activity has been shown to inhibit activation of the limbic system particularly the amygdala (Mayberg 2000; Phan et al 2002). The person conducting the scan was able to communicate with the patient using an audio device through the headphones that the subject wore to decrease effects of scanner noise during the scan. Subjects were asked periodically and specifically before the start and at the end each of the scan how they were doing and whether they were able to follow the instructions for each of the scans. The subjects were asked to respond by moving their feet that were outside the scanner. Scans were only done if the subjects periodically indicated that they were comfortable and able to follow instructions given to them.

The fMRI sequence was as follows:

1. T_1-weighted three axes localizer image: repetition time/echo time (TR/TE) 500/3 msec; Thickness/Gap 5/1.5 mm;

matrix: 256 x 128; field of view (FOV) 24 x 24 cm; 1 number of excitations (NEX); Time 0:30.
2. T1-weighted whole brain image using a 3D magnetization prepared spoiled gradient echo sequence to provide real 1 x 1 x 1 mm^3 spatial resolution. Time 9:37. This scan is necessary to calculate Talairach landmarks in each subject's data in order to permit inter-subject co-registration of results.
3. Anatomic scan: T1-weighted axial images: TR/TE 500/12 msec; 16 slices covering from 5 mm below top of brain inferiorly; Thickness/Gap 7.0/2.0 mm; matrix: 256 x 128; FOV 24 x 24 cm; 1 NEX.
4. fMRI activation scan. 2D Gradient echo EPI sequence: TR/TE 2000/50 msec; matrix: 64 x 64; FOV 24 x 24 cm; flip 90 degrees; bandwidth +/− 62.5 kHz; and 166 repetitions. This scan was acquired with the same slice locations, thickness, and gap as in scan 3. Total scan time for five 1 min picture blocks (please see below) and a 16 second stabilization period at the beginning and end of the scan was 5 min and 32 sec. This scan was repeated 2 times once for the negative versus neutral contrast and a second time for the neutral versus positive contrast (please see below).
5. fMRI LFBF correlations scan. 2D Gradient echo EPI sequence: TR/TE 400/50 msec; 4 transverse slices which included a priori decided upon ROIs - DLPFC, ACC, MTHAL/PST and AMYG identified on the reformatted high resolution SPGR planes (from scan 2) by trained neuroradiology staff (YW and VBM); Thickness = 7 mm, with the gap adjusted to acquire desired slices; matrix: 64 x 64; FOV 24 x 24 cm; flip 30 degrees; bandwidth ± 62.5 kHz; 512 repetitions; Scan time: Time 5 min and 7 sec; repeated 4 times.

Visual Stimulus

The following sequence was created using E-prime software (Psychology Software Tools, Inc, Pittsburgh, Pennsylvania) for paradigm design for presentation of pictorial stimuli.

Activation Scan. Scan 1: negative vs. neutral pictures. Alternate blocks of 4 neutral and 4 negative pictures. Three blocks of neutral and 2 blocks of negative pictures were presented. Each block was presented for 1 minute and each picture was shown for 15 secs. Scan time 5 min and 32 secs. Resting Interval 2 – 5 min.

Scan 2: negative vs. positive pictures. Alternate blocks of 4 positive and 4 negative pictures. Three blocks of negative and 2 blocks of positive pictures were presented. Each block was presented for 1 minute and each picture was shown for 15 secs. Total: Scan time 5 min and 32 secs.

Connectivity Scan. Scan 3: resting state, eyes closed with no task performance. Total scan time: 5 min.

Scan 4: continuous exposure to neutral pictures: 20 neutral pictures (each for 15 sec) Total scan time: 5min.

Scan 5: continuous exposure to positive pictures: 20 positive pictures (each for 15 sec). Total scan time: 5 min.

Scan 6: continuous exposure to negative pictures: 20 negative pictures (each for 15 sec). Total scan time: 5 min.

Image Analysis

Data Reconstruction. This was done using in-house software. The raw image data was Hamming-filtered to improve signal-to-noise ratio with minimal reduction in spatial resolution (Lowe and Sorenson 1997).

Activation fMRI Data Analysis. Reconstructed data were analyzed using SPM 99 image analysis software (Wellcome Department of Cognitive Neurology, London, United Kingdom). EPI images were corrected for motion and differences in slice time acquisition, spatially normalized to MNI (Montreal Neurological Institute) space, and smoothed (8 mm full width half maximum (FWHM)). A boxcar reference function convolved with canonical hemodynamic response function (HRF) was constructed for negative versus neutral and negative versus positive pictures respectively. The first eight images from each trial were discarded to ensure that only image data acquired during MR signal in stable state were analyzed. A high pass filter was used in specification of the boxcar models to remove global low frequency confounds. Then a *t*-contrast map of a single subject was estimated by SPM99. The *t*-contrast maps for each subject were then entered into a second level, random effects analysis. Random effects analysis *t*-contrast maps were then superimposed on SPM/MIN canonical T1 structural scan provided in the database of SPM 99. A nonlinear transform of MNI to Talairach coordinates for each activation area was calculated by a Matlab function provided in SPM99.

The identification of these areas was done using pre-defined anatomical ROI libraries provided within SPM 99 (Tzurio-Mazoyer 2002). The DLPFC ROI was defined as the area within the lateral cortical ribbon of Brodmann's area 9 and 46 which showed the greatest activation at baseline in response to negative versus neutral pictures. For negative versus neutral pictures the percent signal change for each block of pictures was calculated as: ((average signal of all voxels within the region on exposure to negative pictures – average signal of all voxels within the region on exposure to neutral pictures)/ average signal of all voxels within the region on exposure to neutral pictures) x 100. The percent signal change was calculated with a correction for 8 second delay in the onset of hemodynamic response from the start of stimulus presentation.

LFBF Correlation Analysis. Scans were first evaluated for motion. This was done using the "3dvolreg" module in analysis of functional neuroimages (AFNI) (Cox 1996), which uses an iterative least-squares algorithm to determine the variance in a voxel, between images due to motion. No scan was found with a shift of more than .6 mm in any plane or a rotation of more than .6° in any direction and therefore motion correction was not required. The number of slices that can be obtained is limited in the LFBF technique as the sampling rate needs to be set so as to exclude aliasing of cardiac and respiratory related fluctuations in the data. Therefore, a maximum of 4 slices could be obtained corresponding to the ROIs but these slices were noncontiguous. Normalization of these slices in the Talairach space was not attempted as this could potentially lead to significant distortion of the LFBF data. Instead a priori defined ROIs were drawn based on anatomy. Next, the processing for LFBF correlation was done in three steps:

1. Low-pass filtering: Data was corrected for global drifts. Next, data from each pixel were passed through a finite-impulse response (FIR) filter to remove all frequencies above .08 Hz. This removes the oxygenation fluctuations from direct sampling of respiratory and cardiac-related oxygenation fluctuations (Lowe et al 1998).
2. Selection of regions of interest (ROIs): For the purpose of this analysis we decided to focus on the LFBF correlations between the ACC and the MTHAL, AMYG and PST as these are regions which have been most frequently implicated in differ-

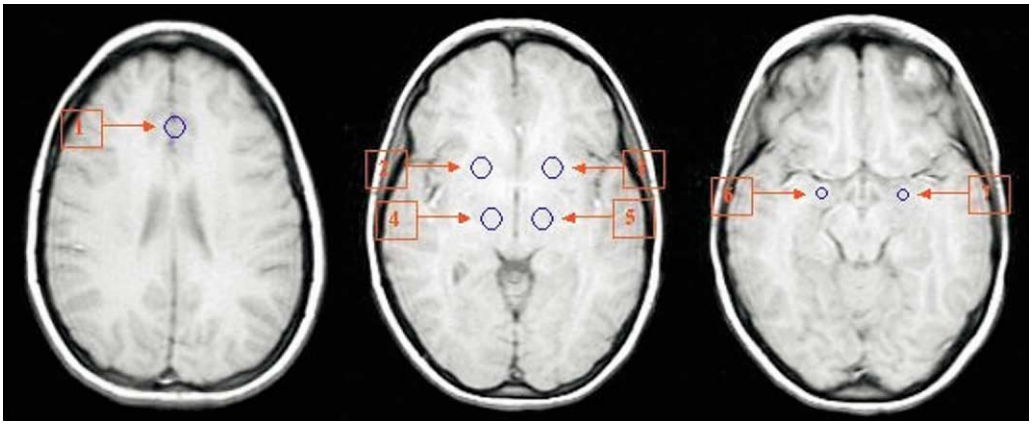

Figure 1. Region of Interest (ROI) placement for sampling of low frequency BOLD fluctuations (LFBF) for cortico-limbic connectivity analysis. 1: anterior cingulate cortex; 2, 3: pallido-striatum (PST); 4, 5: medial thalamus (MTHAL); 6, 7: amygdala (AMYG).

ent studies to be part of the cortico-limbic MRC (Damasio 1997; Drevets 2000a; Mayberg 2000). ROIs were placed by trained radiology staff (YW) in conjunction with a neuroradiologist (AK) corresponding to the a priori defined cortico-limbic areas whose activation was confirmed with the activation scan (Figures 2A and 2B). Anatomically defined region-of interest (ROIs) corresponding to the different regions of the cortico-limbic network as described in published literature were manually traced on each subject's high-resolution anatomy. The center of each ROI was traced based on anatomical landmarks for different areas in the cortico-limbic network, with reference to the activation maps of functional scan from same subject. ACC ROI was delineated in the rostral ACC region (Brodmann area 24A (Phillips et al 2003) as described by Shin and colleagues (Shin et al 2001). The inferior boundary was the intercommissural plane; subgenual portions of ACC (operationally defined here as ACC below $z < 0$) were excluded from this region of interest because our earlier studies demonstrated signal dropout in this region due to susceptibility artifacts as well as data in primates demonstrating that the posterior extent of limbic projections to ACC are located immediately superior to the genu of the corpus callosum, the posterior boundary was defined as the coronal slice 24 mm anterior to the anterior commissure. Striatal-pallidal ROI was defined as reported by Burruss and colleagues (Burruss 2000) and partially covered putamen and lateral palladium. The LFBF slices were 8 mm thick and therefore covered middle and ventral portions of the striato-pallidium. ROI were not placed in the more ventral regions of the striatum due to abrupt decrease in signal in these regions and presence of susceptibility artifacts. Pre-genual ACC was chosen as the ROI as a number of neurological studies have indicated that it is involved in regulation of emotions (Critchley 2004; Damasio 1997; Drevets 1998; Mayberg et al 2000).

Using "draw dataset" function in AFNI (Cox 1996), ROIs were defined as circles with a radius of 4 mm for Amygdala and 6 mm for ACC, medial Thalamus and Striatum ROIs, respectively. Amygdala ROI was smaller than then other regions as this region is anatomically smaller than the other regions being studied. At the same time, the size was determined based on the in-plane resolution of connectivity EPI data to make sure that certain number of voxels (7 for amygdala and 12 for other ROI regions) were selected for further analysis. Those numbers are consistent with previous reports of connectivity study from our institution (Lowe et al 1998).

3. Correlation analysis: For the time series acquired for 512 time points, an average for each time point was calculated for all the pixels within each ROI. This averaged 512 time points series was then corrected for global drift prior to the correlation analysis (Lowe et al 1999). Correlation was calculated between the time series using ACC ROI as a reference ROI with time series of the PST, AMYG and MTHAL ROIs across all time points (512 time points)(Lowe et al 1998). The correlation coefficient was then transformed to a *t* statistic (Lowe et al 1998) to enable comparison between groups. The *t*-score of correlation of LFBF between two ROIs was calculated for each of the time series acquired during resting state and during exposure to neutral, positive and negative pictures, for the depressed patients and healthy controls. LFBF correlation *t* scores between ACC and the limbic regions – AMYG, PST and MTHAL, were used as measures of connectivity between these regions (see Figure 1).

Statistical Analysis

For activation data, statistical analysis was done within SPM 99 as described above. As ROIs of interest were identified a priori areas of activation were examined $p < .005$ (uncorrected) (Friston et al 1995).

For LFBF correlations data, analysis of variance (ANOVA) models were conducted for differences between the two groups (depressed patients and healthy subjects) for each pair of ROIs with the LFBF correlations *t* score as the dependent variable. ANOVA was done for between group effects using LFBF correlation *t* scores between ACC and PST, MTHAL, and AMYG on each side in the resting state and during exposure to neutral, positive and negative pictures. The PROC MIXED procedure in the statistical software package SAS (SAS Institute, Inc., Cary, North Carolina) was used to conduct the analysis, and an unstructured variance-covariance matrix was used for the correlation structure. The significance level of hypothesis testing was set at $p < .05$ (uncorrected) as correlations were calculated between a priori defined ROIs.

Results

Seventeen depressed patients and 17 healthy subjects completed the fMRI scan. Results are presented for 15 depressed and

Table 1. Demographic and Clinical Characteristics of the Sample

	Depressed Patients (n = 15)	Healthy Subjects (n = 15)
Age	28 ± 9	28 ± 7
Sex	11 Female, 4 Male	11 Female, 4 Male
Ethnicity	13 Caucasian, 2 African American	14 Caucasian, 1 African American
25-item HDRS Score	31 ± 8;	NA
Number of Previous Episodes	3 ± 2	NA
Duration of Illness	6 ± 7 years	NA
Drug Free Period	Treatment Naïve – 8 patients Rest of the patients: 24 ± 33 weeks	NA

HDRS, Hamilton Depression Rating Scale. Unless otherwise indicated, data are expressed as mean ± SD.

15 age, sex and ethnicity matched healthy subjects. One patient was excluded because she reported that she was unable to attend to the pictures during the scan. One healthy subject was excluded due to failure of the proper acquisition of fMRI data leading to a warped image. One patient and 1 healthy subject were excluded because there were no close matches in terms of age, sex and ethnicity. Results are presented for 15 depressed and 15 age, sex and ethnicity matched healthy subjects. However, one African American subject in patient group was matched for age and sex but not ethnicity. Subject characteristics are detailed in Table 1 including duration of illness, number of depressive episodes and medication status for the depressed patient group.

Regional Activity Analysis

The negative versus positive picture contrast showed increased activation of the areas of the MRC but did not reach significance levels ($p < .05$) therefore this report will focus mainly on the negative versus neutral contrast.

Group Effects for Response to Negative versus Neutral Pictures. A one way ANOVA for group differences between depressed and healthy subjects revealed that depressed patients had significantly increased activation of the ACC, insula and parahippocampal areas (Figure 2B). A comparison between depressed patients and healthy subjects revealed a greater activation of the AMYG, PST, insula, ACC, and AMPFC in depressed subjects in terms of brain area activated as well as percent signal change (Table 2; Figure 2). MTHAL activation was, however, similar in both groups.

The activation data confirmed our hypothesis that the areas that were designated a priori as the components of the MRC were indeed activated more in response to negative pictures versus neutral pictures in depressed patients compared to healthy controls.

LFBF Correlation Analysis

Resting State. In resting state, LFBF was sampled from the ROIs while subjects' eyes were closed. During resting state cortico-limbic LFBF correlations (i.e. ACC with AMYG, PST and MTHAL) were decreased in depressed patients compared to healthy subjects (Figure 3). The significant decreases were found in ACC-lMTHAL ($p < .01$), ACC-rMTHAL ($p < .02$) and ACC-lPST ($p < .02$) (Table 3).

Neutral Pictures. The differences between depressed patients and healthy subjects seen in the resting state were also seen during exposure to neutral pictures i.e. patients had decreased cortico-limbic LFBF correlations as compared to healthy subjects (Figure 4). Significant differences were found in ACC-lPST ($p < .01$) and ACC-rPST ($p < .01$) LFBF correlations (Table 4).

Positive Pictures. The differences between depressed patients and healthy subjects seen in the resting state were maintained during exposure to positive pictures (i.e. patients had decreased cortico-limbic LFBF correlations as compared to healthy subjects; Figure 4). Significant differences were found in ACC-lMTHAL ($p < .02$), ACC-rMTHAL ($p < .05$) and ACC-rPST ($p < .01$) (Table 4).

Negative Pictures. The differences between depressed patients and healthy subjects seen in the resting state were also

Table 2. Regional Activation on Exposure to Negative versus Neutral Pictures in Depressed Patients (n = 15) and Healthy Controls (n = 15)

			Healthy Controls						Depressed Patients					
			Coordinate[a]			z^c	Extent[b]	%	Coordinate[a]			z^c	Extent[b]	%
ROI	Brodmann Area	Side	x	y	z	Score	(mm^3)	Change	x	y	z	Score	(mm^3)	Change
Amygdala		Left	−24	−7	−20	3.32	80	.12	−28	−5	−18	3.39	96	.16
		Right	26	−7	−15	3.15	56	.09	30	−3	−18	3.41	568	.14
Putamen		Left	−20	−1	11	3.23	176	.03	−22	−10	6	4.36	1,512	.12
		Right	24	1	11	3.65	632	.03	24	0	15	3.3	1,200	.11
Thalamus		Left	−10	−14	1	5.89	7,824	.11	−14	−10	4	4.03	6,872	.12
		Right	10	−14	1	4.08	5,736	.09	14	−11	4	3.39	5,256	.11
DLPFC	9	Left	−44	13	27	4.75	600	.03	−44	−13	29	3.42	592	.06
	9	Right	50	17	25	4.56	1,968	.08	46	9	28	4.32	4,680	.10
AMPFC	9/8	Left	−6	56	30	4.31	5,376	.07	−8	46	43	6.79	11,008	.19
	9/8	Right	4	56	30	3.95	3,108	.04	14	45	44	6.05	5,528	.14
Insula	13	Left	−44	−11	14	−4.21	184	−.02	−38	14	6	3.22	2,016	.10
	13	Right	46	−7	12	−3.67	120	−.02	44	21	−2	3.07	112	.11
Anterior Cingulate	32	Right	10	38	20	−3.56	1,168	−.06					0	.06
Middle Cingulate	24	Left	−2	−29	40	−4.78	2,560	−.05	−10	21	34	3.66	1,024	.06
	24	Right	14	2	44	−3.44	1,600	−.05	8	−10	30	3.93	960	.07

ROI, Region Of Interest; AMPFC, Anteromedial Prefrontal Cortex; DLPFC, Dorsolateral Prefrontal Cortex.
[a]Coordinates are those pixels in activation ROI, which have maximum z-scores: Coordinates are in Talairach space.
[b]Extent (mm^3): Total area of all voxels for which activation was significant at $P < .005$.
[c]For z scores ≥ 2.98, $p < .005$; for $z \geq 3.79$, $p < .001$; for $z \geq 4.14$, $p < .0005$; for $z \geq 4.99$, $p < .0001$.

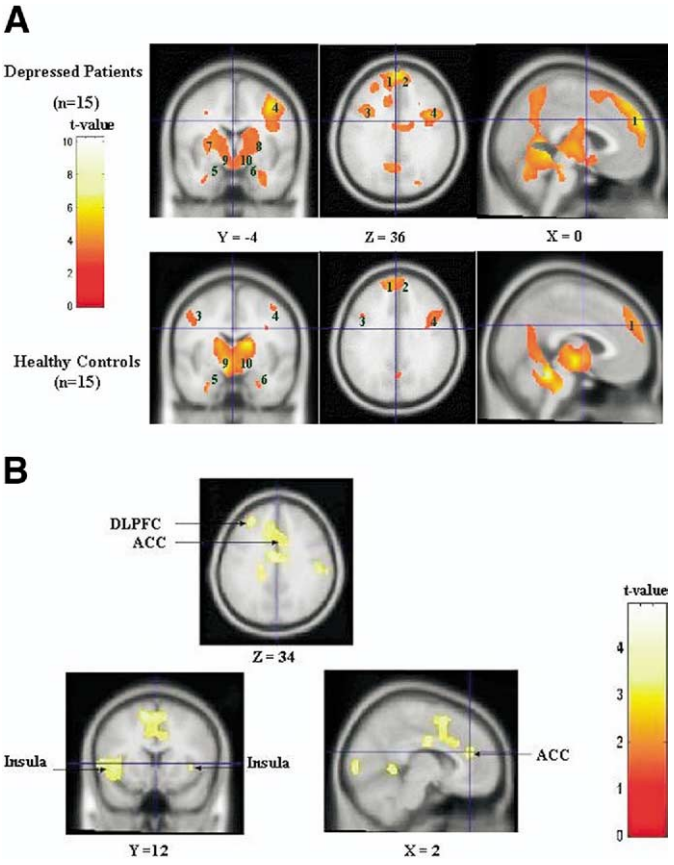

Figure 2. (A) Regional brain activation in response to negative versus neutral pictures in depressed patients and healthy subjects. Coordinators are in MNI space. 1: Anteromedial Pre-frontal cortex (AMPFC)(L); 2: AMPFC (R); 3: Dorsolateral prefrontal cortex (DLPFC)(L); 4: DLPFC (R); 5: Amygdala (L); 6: Amygdala (R); 7: Putamen (L); 8: Putamen (R); 9: Thalamus (L); 10: Thalamus (R). $p < .005$. **(B)** One-way analysis of variance (ANOVA) for difference in activation between patients and healthy controls in response to negative versus neutral pictures. Coordinates are in Montreal Neurological Institute (MNI) space. Patient group versus control group before treatment ($n = 15$; $p < .003$). ACC, anterior cingulate cortex.

seen during exposure to negative pictures (i.e. patients had decreased cortico-limbic LFBF correlations as compared to healthy subjects; Figure 4). However, significant differences were found only for ACC-rPST ($p < .03$) LFBF correlations (Table 4). In both depressed patients and healthy subjects, ACC-AMYG LFBF correlations were decreased on exposure to negative pictures compared to neutral pictures (Figure 4) however this decrease did not reach significance levels.

Discussion

Activation data from this study confirmed results from previous reports that depression is associated with increased activation of limbic regions such as the AMYG, PST, MTHAL, and insula. In this study, cortical components of the MRC such as the AMPFC, DLPFC and ACC also showed increased activation in response to negative versus neutral stimuli. Other fMRI studies have also reported increased activation of these areas to be related to evoked sadness in healthy subjects (Teasdale et al 1999) and to treatment response in depression (Davidson et al 2003). Greater activation of cortical regulatory areas such as the ACC may be the result of greater activation required to regulate

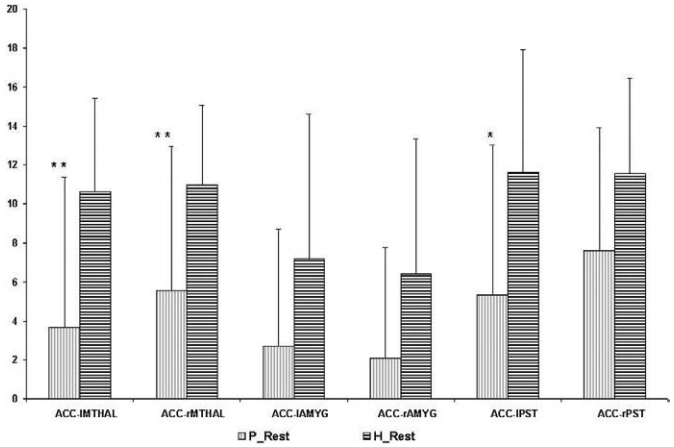

Figure 3. Resting state cortico-limbic LFBF correlations (mean ± SEM) in unmedicated depressed patients ($n = 15$) and healthy control subjects ($n = 15$). LFBF, low frequency BOLD fluctuations; ACC, anterior cingulate cortex; LMTHAL, left medial thalamus; RMTHAL, right medial thalamus; LAMYG, left amygdala; RAMYG, right amygdala; LPST, left pallido-striatum; RPST, right pallido-striatum; P Rest, patients resting state; H Rest, healthy control subjects resting state. ** $p < .01$; * $p < .05$.

the abnormal limbic activation on exposure to negative stimuli versus neutral stimuli. Increased activation of the anteromedial prefrontal cortex (AMPFC) (Area 9) has also been reported in several other studies (Teasdale et al 1999). It has been suggested that cortical areas such as the AMPFC which show increased activation with negative versus neutral stimuli in fMRI studies may be involved in the processing of affect related meanings of stimuli (Teasdale et al 1999).

Blood flow and regional glucose metabolism studies using PET have reported decreased activation of the prefrontal cortical regions in depression. However, this decreased activation or inhibition of activation cannot be measured using fMRI because analysis is based on the contrast between changes in blood flow on exposure to task of interest (in this case: negative pictures) versus a control task (in this case: neutral pictures).

The pallidostriatum is thought to be an integral part of the MRC and both structural and functional abnormalities have been

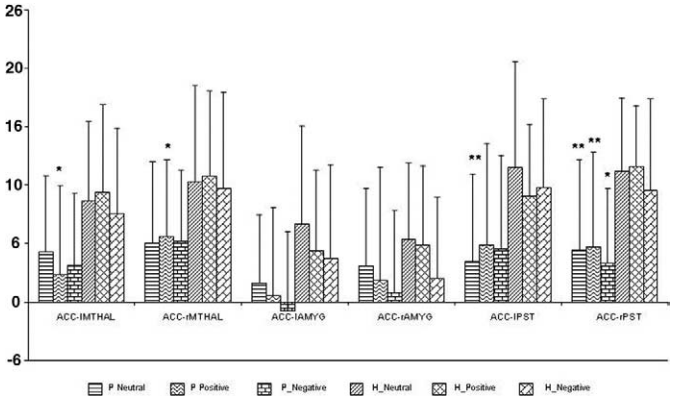

Figure 4. Cortical-limbic LFBF correlations on exposure to neutral, positive, and negative pictures in depressed patients ($n = 15$) and healthy subjects ($n = 15$). LFBF, low frequency BOLD fluctuations; ACC, anterior cingulate cortex; LMTHAL, left medial thalamus; RMTHAL, right medial thalamus; LAMYG, left amygdala; RAMYG, right amygdala; LPST, left pallido-striatum; RPST, right pallido-striatum; P, patients; H, healthy control subjects. ** $p < .01$; * $p < .05$.

Table 3. Differences of Connectivity (LFBF Correlations) *t*-scores Between Patients ($n = 15$) and Healthy Subjects ($n = 15$) in Resting State

	Patient vs. Healthy		
$\Delta_{t\text{-score}}$	$\Delta_{resting}$[a]	*t*-Value	*p*-Value
Cortical-Limbic			
ACC-lMTHAL	−6.94 (2.3)	−2.98	.01[b]
ACC-rMTHAL	−5.43 (2.2)	−2.48	.02[b]
ACC-lAMYG	−4.48 (2.5)	−1.82	.08
ACC-rAMYG	−4.34 (2.3)	−1.87	.07
ACC-lPST	−6.30 (2.6)	−2.45	.02[b]
ACC-rPST	−3.94 (2.1)	−1.90	.07

ACC, Anterior Cingulate Cortex; PST, Pallido-Striatum; AMYG, Amygdala; MTHAL, Medial Thalamus; LFBF, low frequency BOLD fluctuations.
[a]Mean (Std. Err).
[b]Significant level at 0.05, p-values were defined based on DF = 28.

reported in mood disorders. In particular, subcortical dysfunction has been related to motor and cognitive abnormalities in depression. Basal ganglia-thalamocortical circuits are thought to be parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions and medial thalamus is involved in emotional perception and regulation (Alexander et al 1990; Phillips et al 2003). The amygdala has been shown to be central to the brain's response to noxious stimuli (Adolphs 1999; Irwin et al 1996; LeDoux 2000) and has been shown to be activated in a number of studies in depression (Drevets et al 1992; Phillips et al 2003). Therefore, regional activation abnormalities are expected in these regions and our findings are consistent with reported abnormalities in the literature.

Cortico-limbic LFBF correlations showed a decrease in depressed patients compared to healthy subjects. The cortico-limbic LFBF correlations results indicate possible decreased phase coherence between LFBF sampled in the ACC and the limbic regions PST and MTHAL in depressed patients. This decreased phase coherence between LFBF in the ACC and the limbic regions may be associated with decreased cortico-limbic connectivity in depression. Decreased cortico-limbic connectivity could lead to a decreased regulatory effect of ACC over the limbic areas leading to emotional dysregulation which is thought to be the hallmark of the depressed state.

The activation experiment was analyzed after the fMRI scan was completed, but for the connectivity scan the four noncontiguous slices had to be identified in the anatomical scan while the patients were in the scanner which would include the a priori decided upon regions of interest. ROIs, based on a priori decided upon regions of interest, were placed on the anatomical slices for which LFBF data were acquired and may not have exactly corresponded to the areas seen to be activated in the activation analysis. However, a comparison of the activation analysis and the ROI placement on the connectivity scan showed that the activation areas in the AMYG, PST and MTHAL were broadly in the same regions where ROIs were placed for the LFBF correlation analysis.

This study was limited by the small number of subjects studied for the number of variables analyzed, particularly for the cortico-limbic LFBF correlations data. For this study we had a priori defined ROIs and had an a priori hypothesis that cortico-limbic connectivity will be decreased in depression. Therefore, the LFBF correlations results which are congruent with our a priori hypothesis could be interpreted without a correction for multiple correlations. However, as a lower threshold of significance was used for connectivity analysis, the results of this study should be considered as provisional until future studies, with larger number of number of patients for greater statistical power, confirm these findings.

Differences between depressed patients and healthy subjects could be due to differences in anxiety levels rather than depression. Adequate education about the procedure verbally and through pictures, and adequate preparation of the subject before starting the scan was done. The IAPS pictures that were chosen were specifically selected to have low arousal scores.

The lower contrast between positive and negative pictures may indicate that the fMRI activation changes seen were not specific to type of emotion. Positive and negative stimuli have been shown to activate similar subcortical brain areas (Liberzon et al 2003). Furthermore, depressed patients have been shown to react to positive pictures in a negative way (Kumari et al 2003) and this could be another reason for the decreased negative versus positive picture contrast seen in this study.

The activation of the insula may suggest that general arousal rather than quality of stimulus may be the cause of the regional activation seen. However, we chose only those IAPS pictures which specifically had low arousal scores as rated by a large normative population sample (Lang et al 1997). Furthermore, insula activation has been shown to be sensitive to noxious stimuli and its activation may be important in appraisal of the negative salience of a stimulus (Phillips et al 2003). However, the

Table 4. Differences of Connectivity (LFBF Correlations) *t*-scores Between Patients ($n = 15$) and Healthy Subjects ($n = 15$) on Exposure to Neutral, Positive and Negative Pictures

	Neutral			Positive			Negative		
ROIs	Difference in *t*-Score[a]	*t*-Value	*p*-Value	Difference in *t*-Score[a]	*t*-Value	*p*-Value	Difference in *t*-Score[a]	*t*-Value	*p*-Value
Cortical-Limbic									
ACC-lMTHAL	−4.35 (2.4)	−1.80	.08	−7.05 (2.8)	−2.55	.02[b]	−4.44 (2.5)	−1.79	.08
ACC-rMTHAL	−5.27 (2.8)	−1.89	.08	−5.20 (2.5)	−2.04	.05[b]	−4.57 (2.6)	−1.74	.09
ACC-lAMYG	−5.10 (2.7)	−1.92	.07	−3.80 (2.6)	−1.44	.16	−4.46 (2.7)	−1.64	.11
ACC-rAMYG	−2.32 (2.4)	−.97	.34	−3.01 (3.1)	−.98	.34	−1.22 (2.5)	−0.48	.64
ACC-lPST	−8.02 (3.0)	−2.63	.01[b]	−4.13 (2.7)	−1.50	.14	−5.27 (2.9)	−1.85	.08
ACC-rPST	−6.68 (2.6)	−2.60	.01[b]	−6.84 (2.5)	−2.77	.01[b]	−6.20 (2.6)	−2.36	.03[b]

ACC, Anterior Cingulate Cortex; PST, Pallido-Striatum; AMYG, Amygdala; MTHAL, Medial Thalamus; LFBF, low frequency BOLD fluctuations.
[a]Mean (Std. Err).
[b]Significant level at .05, *p*-values were defined based on $df = 28$.

effects of arousal on the fMRI measures used in this study cannot be completely ruled out.

In the connectivity scan there was no picture effect in terms of decrease in connectivity on exposure to positive and negative pictures in depressed patients. Healthy subjects did show a decrease in corticolimbic connectivity on exposure to negative pictures but it did not reach significance levels. However, this effect was manifest in the decreased difference between depressed and healthy groups during continuous exposure to negative pictures. This suggests that a floor effect may be present for corticolimbic connectivity in depressed patients.

We purposely did not ask subjects to rate the pictures so as not to introduce a significant cognitive component which may interfere and decrease activation of the limbic regions (Hariri et al 2003; Phan et al 2002). Consequently, we were also not able to obtain a behavioral measure of response to the pictures. This is an inherent problem in the technique of passive exposure to stimulus in fMRI and has to be balanced with the confounding effects of introducing a cognitive task (Phan et al 2002). In this study, we elected to minimize the latter. This strategy seems to have been successful as we obtained significant activation of the limbic regions, particularly the amygdala. In future studies, other techniques could be used to obtain behavioral ratings without interfering with activation secondary to emotional response (e.g., by introducing a resting state between scans in which ratings for the block just presented could be obtained or by obtaining ratings after the scan). However, the latter technique, which has been used by some studies, is limited by its retrospective nature and also by the fact that only conscious emotional responses are reported.

An order effect may be present in the cortico-limbic LFBF correlations data as pictures were always shown in the same sequence. However, it has been shown that negative pictures can contaminate the effects of neutral pictures much more than the other way around. Therefore, negative pictures were shown last (Davidson et al 2003). There was a time delay of a few min between blocks of fMRI acquisition. For activation scan the first and the last 8 acquisitions and for the cortico-limbic LFBF correlations scan the first 50 acquisitions were discarded. Therefore, contamination effects were minimized but cannot be completely ruled out. In future studies, a resting state could be introduced between each type of picture block to further minimize contamination effects. The amygdala and possibly other areas of the brain are susceptible to habituation during the continuous exposure to a stimulus. This technique would also be helpful in decreasing effects of habituation to the stimulus in the amygdala.

Finally, the ventral areas of the brain such as the amygdala are characterized by lower signal to noise ratio than from cortical areas because of susceptibility artifacts. An adequate signal for analysis was also not obtainable for ventral regions of the brain such as the ventral striatum including the nucleus accumbens and the subgenual cingulate cortex. In future studies, techniques such as z –shimming, spiral acquisition (Li et al 2003) or coronal pseudooblique slice (Chen and Wyrwicz 2001) techniques could be used to decrease susceptibility artifacts in the ventral regions of the brain.

The observed abnormalities of increased cortico-limbic activation in response to negative stimuli and decreased LFBF correlations between the ACC and PST and MTHAL seen could be either a state or a trait effect. To test out this difference, depressed patients need to be studied after treatment when they are not depressed, or unmedicated euthymic patients with a previous history of depression need to be studied. In an extension of this study, we investigated the effect of antidepressant treatment and will report the results in a separate manuscript. After treatment with an antidepressant, abnormalities of activity and connectivity seen in the baseline state do show a reversal and therefore some of these abnormalities are likely to be state related (Anand et al, in press).

A number of studies have reported structural abnormalities in patients with depression including decreased volume of cortical regions such as the prefrontal and orbitofrontal cortex and the limbic areas such as amygdala and hippocampus (Bremner 2002; Sheline et al 1996, 1998). White matter abnormalities have also been reported using diffusion tensor imaging (DTI) techniques (Taylor et al 2004). In future studies, an analysis of the relationship between functional connectivity and structural abnormalities will be useful in understanding the pathophysiology of depression.

The neurophysiological basis of LFBF in steady state data is thought to be related to neuronal firing in the resting state (Maldjian 2001) but still remains to be fully clarified. In traditional fMRI activation studies, LFBF are excluded as low frequency noise using filtration techniques. Cardiac and respiratory rhythms as well as head motion can create artifacts which can confound LFBF analysis. However, with proper techniques, using sampling rates and frequency range to exclude the effects of physiological cycles and minimize motion, LFBF measurement which is independent of these confounds can be obtained (Lowe et al 2000). Further animal studies need to be done to investigate the origin of LFBF and its relationship to baseline neuronal firing. At present, most of the LFBF studies have been done in healthy subjects to study normal brain physiology. Further work needs to be done to investigate the use of LFBF techniques to study abnormalities in neuropsychiatric disorders to test its validity. In this regard, changes in LFBF correlation as a measure of abnormal connectivity have been reported in disease states such as multiple sclerosis (Lowe et al 2002), and in brief reports in schizophrenia (Driesen, unpublished data), depression (Skudlarski et al 2000), and bipolar disorder (Blumberg, unpublished data). Therefore, LFBF correlation methods could be used to study brain connectivity in neuropsychiatric illness and treatment effects and further work needs to be done in this area.

Keeping the above caveats in mind, the results of this study indicate that increased activation of cortical regions such as the AMPFC and limbic regions such as the AMYG, PST and MTHAL is present in depression and provides evidence for possible decreased connectivity between the ACC and limbic regions in depressed patients compared to healthy control subjects. The methodology used in this study could also be used in future studies to characterize circuit level abnormalities in other psychiatric illnesses such as schizophrenia and obsessive compulsive disorder and investigate the effects of pharmacological treatment in these disorders.

The results of this study have been previously presented in part at the Meeting of Society of Biological Psychiatry and American Psychiatric Association Meeting, San Francisco, May, 2003; and at Human Brain Mapping Meeting, New York, June, 2003.

Jacqueline Zimmerman assisted in MRI scanning. Mamata

Kamat assisted in manuscript preparation. Shirley Jennings and Cyndi Li assisted in coordination of the project. This study was supported by Indiana University 21st Century Fund and Indiana Genomics Initiative fund.

Adolphs R (1999): The human amygdala and emotion. *The Neuroscientist* 5:125–137.

Alexander GE, Crutcher MD, DeLong MR (1990): Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Progress in Brain Research* 85:119–46.

Anand A, Charney DS (1999): Abnormality of catecholamines and pathophysiology of bipolar disorder. In: Soares JC, Gershon S, editors. *Bipolar Disorder: Basic Mechanisms and Therapeutic Implications*. New York: Marcel Dekker, 59–94.

Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, et al (in press): Antidepressant effect on connectivity of mood regulating circuit. *Neuropsychopharmacology*.

Arfanakis K, Cordes D, Haughton VM, Moritz CH, Quigley MA, Meyerand ME (2000): Combining independent component analysis and correlation analysis to probe interregional connectivity in fMRI task activation datasets. *Magnetic Resonance Imaging* 18:921–30.

Biswal BB, Yetkin FZ, Haughton VM, Hyde JS (1995): Functional connectivity in the motor cortex of resting human brain. *Magnetic Resonance in Medicine* 34:537–541.

Bremner JD (2002): Sturctural changes in the brain in depression and relationship to symptom recurrence. *CNS Spectrums* 7:120–139.

Burruss JW (2000): Functional neuroanatomy of the frontal lobe circuits. *Radiology* 214:227–230.

Chen NK, Wyrwicz AM (2001): Optimized distortion correction technique for echo planar imaging. *Magnetic Resonance in Medicine* 45:525–8.

Cox RW (1996): AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29:162–73.

Critchley HD (2004): The human cortex responds to an interoceptive challenge. *Proc Natl Acad Sci U S A* 101:6333–4.

Damasio AR (1997): Towards a neuropathology of emotion and mood. *Nature* 386:769–770.

Davidson RJ, Irwin W, Anderle MJ, Kalin NH (2003): The neural substrates of affective processing in depressed patients treated with venlafaxine. *American Journal of Psychiatry*. 160:64–75.

Drevets WC (1998): Functional neuroimaging studies of depression: the anatomy of melancholia. *Annual Review of Medicine* 49:341–61.

Drevets WC (2000a): Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Progress in Brain Research* 126:413–31.

Drevets WC (2000b): Neuroimaging studies of mood disorders. *Biological Psychiatry* 48:813–29.

Drevets WC, Price JL, Simpson JR Jr., Todd RD, Reich T, Vannier M, et al (1997): Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386:824–7.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992): A functional anatomical study of unipolar depression. *Journal of Neuroscience* 12:3628–41.

Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1993): Functional connectivity: the principal-component analysis of large (PET) data sets. *Journal of Cerebral Blood Flow & Metabolism* 13:5–14.

Friston KJ, Holmes A, Poline J-B, Price CJ, Frith CD (1995): Detecting Activations in PET and fMRI: Levels of Inference and Power. *Neuroimage* 4:223–225.

Greicius MD, Krasnow B, Reiss AL, Menon V (2003): Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* 100:253–8.

Hampson M, Peterson BS, Skudlarski P, Gatenby JC, Gore JC (2002): Detection of functional connectivity using temporal correlations in MR images. *Human Brain Mapping* 15:247–62.

Hariri AR, Mattay VS, Tessitore A, Fera F, Weinberger DR (2003): Neocortical modulation of the amygdala response to fearful stimuli. *Biological Psychiatry* 53:494–501.

Irwin W, Davidson RJ, Lowe MJ, Mock BJ, Sorenson JA, Turski PA (1996): Human amygdala activation detected with echo-planar functional magnetic resonance imaging. *NeuroReport* 7:1765–9.

Ketter TA (1996): Functional brain imaging, limbic function, and affective disorders. *Neuroscientist* 2:55–65.

Kumari V, Mitterschiffthaler MT, Teasdale JD (2003): Neural abnormalities during cognitive generation of affect in treatment resistant depression. *Biological Psychiatry* 54:777–791.

Lang PJ, Bradley MM, Cuthbert BN (1997): *International Affective Picture System (IAPS): technical manual and affective ratings*. Gainsville: The Center for Research and Psychophysiology, University of Florida.

Lawrie SM, Buechel C, Whalley HC, Frith CD, Friston KJ, Johnstone EC (2002): Reduced frontotemporal functional connectivity in schizophrenia associated with auditory hallucinations. *Biological Psychiatry* 51:1008–11.

LeDoux JE (2000): Emotion circuits in the brain. *Annual Review of Neuroscience* 23:155–84.

Li T-Q, Takahashi A, Wang Y, Mathews VP, Glover GH (2003): Minimizing susceptibility artifacts in BOLD fMRI using 3D dual-echo spiral in (DSPIN) acquisition. *Proc Int Soc Magn Reson Med* Toronto, 738.

Liberzon I, Phan L, Decker LR, Taylor SF (2003): Extended Amygdala and Emotional Salience: A PET Activation Study of Positive and Negative Affect. *Neuropsychopharmacology* 28:726–733.

Lowe MJ, Dzemidzic M, Lurito JT, Mathews VP, Phillips MD (2000): Correlations in low-frequency BOLD fluctuations reflect cortico-cortical connections. *Neuroimage* 12:582–7.

Lowe MJ, Mock BJ, Sorenson JA (1998): Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 7:119–32.

Lowe MJ, Phillips MD, Lurito JT, Mattson D, Dzemidzic M, Mathews VP (2002): Multiple sclerosis: low-frequency temporal blood oxygen level-dependent fluctuations indicate reduced functional connectivity initial results. *Radiology* 224:184–92.

Lowe MJ, Sorenson JA (1997): Spatially filtering functional magnetic resonance imaging data. *Magnetic Resonance in Medicine* 37:723–9.

Maldjian JA (2001): Functional connectivity MR imaging: fact or artifact? *[comment]*. Ajnr: American Journal of Neuroradiology 22:239–40.

Mayberg H (2000): Depression. *In Mazziotta J, Toga AW, Frackowiak R (eds),* Brain Mapping - The Disorders. *London, UK: Academic Press* pp 485–507.

Mayberg HS (1997): Limbic-cortical dysregulation: a proposed model of depression. *Journal of Neuropsychiatry & Clinical Neurosciences* 9:471–81.

Mayberg HS (2002): Modulating limbic-cortical circuits in depression: targets of antidepressant treatments. *Seminars in Clinical Neuropsychiatry* 7:255–68.

Mayberg HS (2003): Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *British Medical Bulletin* 65:193–207.

Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, et al (2000): Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biological Psychiatry* 48:830–43.

Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, et al (1999): Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *American Journal of Psychiatry* 156:675–82.

McIntosh AR, Grady CL, Ungerleider LG, Haxby JV, Rapoport SI, Horwitz B (1994): Network analysis of cortical visual pathways mapped with PET. *Journal of Neuroscience* 14:655–66.

Menon V, Anagnoson RT, Glover GH, Pfefferbaum A (2001): Functional magnetic resonance imaging evidence for disrupted basal ganglia function in schizophrenia. *American Journal of Psychiatry* 158:646–9.

Meyer-Lindenberg A, Poline JB, Kohn PD, Holt JL, Egan MF, Weinberger DR, et al (2001): Evidence for abnormal cortical functional connectivity during working memory in schizophrenia. *American Journal of Psychiatry* 158:1809–17.

Phan KL, Wager T, Taylor SF, Liberzon I (2002): Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*. 16:331–48.

Phillips ML, Drevets WC, Rauch SL, Lane R (2003): Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biological Psychiatry* 54:504–14.

Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, et al (2004): Limbic-frontal circuitry in major depression: a path modeling meta-analysis. *Neuroimage* 22:409–18.

Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA (2001): Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biological Psychiatry* 50:651–8.

Sheline YI, Gado MH, Price JL (1998): Amygdala core nuclei volumes are decreased in recurrent major depression. *NeuroReport* 9:2023–8.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996): Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences of the United States of America* 93:3908–13.

Shin LM, Whalen PJ, Pitman RK, Bush G, Macklin ML, Lasko NB, et al (2001): An fMRI study of anterior cingulate function in posttraumatic stress disorder. *Biological Psychiatry* 50:932–42.

Shulman RG (2001): Functional imaging studies: linking mind and basic neuroscience. *American Journal of Psychiatry* 158:11–20.

Siegle GJ, Steinhauer SR, Thase ME, Stenger VA, Carter CS (2002): Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biological Psychiatry* 51:693–707.

Skudlarski P, Fulbright R, Gore J, Wexler BE (2000): Emotions changes the functional connectivity measured by the fMRI time-course correlations. *Neuroimge* 1:S246.

Stephan KE, Magnotta VA, White T, Arndt S, Flaum M, O'Leary DS, et al (2001): Effects of olanzapine on cerebellar functional connectivity in schizophrenia measured by fMRI during a simple motor task. *Psychological Medicine* 31:1065–1078.

Taylor WD, MacFall JR, Payne ME, McQuoid DR, Provenzale JM, Steffens DC, et al (2004): Late-life depression and microstructural abnormalities in dorsolateral prefrontal cortex white matter. *Am J Psychiatry* 1293-1296.

Teasdale JD, Howard RJ, Cox SG, Ha Y, Brammer MJ, Wiliams SCR, et al (1999): Functional MRI study of the cognitive generation of affect. *American Journal of Psychiatry* 156:209–215.

Thase ME, Carpenter L, Kupfer DJ, Frank E (1991): Clinical significance of reversed vegetative subtypes of recurrent major depression. *Psychopharmacology Bulletin* 27:17–22.

Tzurio-Mazoyer N (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomic parcellation of the MNI MRI single subject brain. *Neuroimage* 15:273–289.