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Evidence for deficient modulation of amygdala response by prefrontal cortex in bipolar mania

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Abstract

Several studies have implicated the involvement of two major components of emotion regulatory networks, the ventrolateral prefrontal cortex (VLPFC) and amygdala, in the pathophysiology of bipolar disorder. In healthy subjects, the VLPFC has been shown to negatively modulate amygdala response when subjects cognitively evaluate an emotional face by identifying and labeling the emotion it expresses. The current study used such a paradigm to assess whether the strength of this modulation was altered in bipolar subjects when manic. During functional magnetic resonance imaging (fMRI), nine manic subjects with bipolar I disorder and nine healthy subjects either named the emotion shown in a face by identifying one of two words that correctly expressed the emotion (emotion labeling task) or matched the emotion shown in a face to one of two other faces (emotion perception task). The degree to which the VLPFC regulated amygdala response during these tasks was assessed using a psychophysiological interaction (PPI) analysis. Compared with healthy subjects, manic patients had a significantly reduced VLPFC regulation of amygdala response during the emotion labeling task. These findings, taken in context with previous fMRI studies of bipolar mania, suggest that reductions in inhibitory frontal activity in these patients may lead to an increased reactivity of the amygdala.

Keywords: Bipolar disorder; fMRI; Functional connectivity; Emotion regulation

1. Introduction

Bipolar mania is characterized by euphoric or irritable mood, distractibility, increased impulsivity and decreased need for sleep (American Psychiatric Association, 1994). Patients in this acute mood state often engage in behaviors with an intensity that can negatively impact both personal and professional aspects of life, and that can have a high potential for harmful consequences.

Very few functional magnetic resonance imaging (fMRI) studies of bipolar mania exist, likely due in part to the difficulty of scanning these subjects. Of these few, however, there is a striking convergence of findings demonstrating deficits in function localized to the

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ventrolateral prefrontral cortices (VLPFC; BA47) during the performance of behavioral inhibition tasks. In a group of 11 manic subjects scanned during a Go/No-Go task, we recently reported a decreased activation of right VLPFC and left anterior cingulate (ACC, BA24) (Altshuler et al., 2005b) relative to control subjects. These results are consistent with a study by Elliott et al. (2004) who reported reduced activation of bilateral VLPFC in a set of eight manic patients using a modified affective version of the Go/No-Go task. Moreover, using a different neurocognitive paradigm, the Stroop interference task, Blumberg et al. (2003) reported decreased activation in left VLPFC among manic patients compared with controls and decreased activation in right VLPFC among manic patients compared with euthymic and depressed bipolar patients. PET studies have similarly pointed to a role for frontal cortex in bipolar mania (Blumberg et al., 1999; Rubinsztein et al., 2001), as have non-imaging lesion studies (Starkstein and Robinson, 1997). Together these reports suggest that dysfunction in the VLPFC, a brain region which normally subserves inhibition and executive control processes, might be one of several areas that could contribute to the behavioral symptoms of mania.

Evidence from healthy individuals has further elucidated the role of the VLPFC to include its involvement in the regulation of emotional aspects of behavior. In 17 healthy subjects, Hariri et al. (2000) found that linguistic processing of emotional faces (naming or labeling the emotion displayed by a face) in contrast to viewing of faces (matching two faces based on the displayed emotion), was associated with an increase in the response of right VLPFC and a simultaneous decrease in the response of the amygdala. Ventrolateral prefrontal regions may thus work to modulate (i.e. inhibit) amygdala activity, forming a system in which people control and direct their emotional responses through the cognitive evaluation of their emotional experiences. Additional support for Hariri's finding that ventrolateral or orbitolateral prefrontal brain regions may act to modulate amygdala activity comes from subsequent studies which used different types of affective stimuli (Hariri et al., 2003; Lieberman et al., 2005, 2007), and studies wherein subjects cognitively reappraised pictures (Ochsner et al., 2002; Phan et al., 2005) or engaged in the effortful suppression of negative emotional experiences (Ochsner et al., 2004). In this regard, it is interesting that of the few fMRI studies of bipolar mania which used activation paradigms to target the amygdala, a significant increase in activity of this structure has been reported among patients vs. control subjects (Altshuler et al., 2005a; Chen et al., 2006; although not all studies have found this; see Lennox et al., 2004).

In the present study, we employed an affective faces paradigm to (1) examine whether VLPFC deficits, previously observed in bipolar-manic subjects during the performance of traditional behavioral inhibition tasks, persist when these patients identify and label an emotional stimulus, and (2) determine whether these deficits correspond with a reduced inhibition of the amygdala. As in Hariri et al. (2000), subjects were required to either actively view affective stimuli (emotion perception task), or assign verbal descriptors to these stimuli (emotion labeling task). We have previously shown that increased activation of the left amygdala occurs during the emotion perception task in a set of nine manic patients (Altshuler et al., 2005a). Using this same group of patients, here we evaluated group differences in brain activation that occur during the verbal labeling of these stimuli, and the inverse coupling between VLPFC and amygdala activity that occurs as a result of this psychological process. Specifically, the modulatory interaction between VLPFC and amygdala was determined using a functional connectivity analysis (Hariri et al., 2000; Horwitz, 2003). This method assesses the degree to which activity in one brain region (i.e. VLPFC) is associated with activity in other regions (i.e. amygdala) in either a negative (i.e., likely inhibitory) or positive (i.e., likely contributory) manner. Based on evidence for decreased VLPFC function in mania, and data suggesting that this region modulates amygdala activity, we hypothesized that compared to healthy subjects, bipolar-manic patients would show less VLPFC activity as well as decreased negative functional connectivity between VLPFC and amygdala during the cognitive evaluation of affective stimuli.

2. Methods

2.1. Subjects

The study protocol was approved by the Institutional Review Board at UCLA and the VA Greater Los Angeles Healthcare System. Each subject gave written informed consent. Manic subjects with bipolar I disorder (history of manic episode) were recruited through the UCLA Mood Disorders Clinic, the Bipolar Disorders Clinic of the Veterans Affairs Greater Los Angeles Health Care System, and the inpatient units of both hospitals. Subjects enrolled in other research projects of the UCLA Mood Disorders Research Program were also invited to participate. Control subjects were recruited by advertisement in local newspapers and campus flyers. Both control and patient populations were evaluated using the Structured Clinical Interview for DSM-IV (SCID) to confirm an accurate diagnosis or absence thereof. Illness duration and medication information for

patients was obtained by patient self report and by reference to medical records, when available. Exclusion criteria for all subjects included left-handedness, hypertension, neurologic illness, metal implants, and a history of skull fracture or head trauma with loss of consciousness >5 min. Exclusion criteria for healthy subjects included current or past psychiatric diagnosis (including history of substance abuse) or current medications. Bipolar subjects with other active Axis I comorbidities were also excluded.

Scans from nine subjects (6 women, mean age=34.6±8.0 years) with bipolar I disorder, currently manic or hypomanic, and nine control subjects (6 women, mean age=30.4±7.6 years) were included in our analysis. The SCID was used to determine whether bipolar subjects met criteria for a current manic episode. Mood symptoms in bipolar subjects were rated on the day of scanning using the Young Mania Rating Scale (YMRS; Young et al., 1978) and the 21-item Hamilton Depression Rating Scale (Hamilton, 1960) to assess for current severity of mania and depression (dysphoric mania). In total, 15 manic/hypomanic bipolar subjects were scanned using this paradigm. Due to excessive motion in six patients, however, data from these subjects were excluded from analysis. The remaining nine patients were ill for 14.8±5.1 years, had mean YMRS scores of 15.1±3.7, HAM-D scores of 9.1±5.3, and had a prior history of 4.2±2.0 manic episodes. Two of the nine bipolar subjects were on no medications at the time of scanning. The remaining seven subjects were currently taking one or more medications to treat their mania, including divalproex sodium (n=4), lithium (n=2), gabapentin (n=2) and olanzapine (n=1).

2.2. Experimental paradigm

The face matching paradigm has been detailed elsewhere (Hariri et al., 2000). Briefly, subjects were presented with three different experimental conditions: "perceive emotion", "label emotion" and the control condition. In the "perceive emotion" condition, subjects matched one of two affectively charged faces at the bottom of the screen to a target face at the top of the screen based on the type of emotional expression (Fig. 1A). In the "label emotion" condition, subjects chose one of two presented words on the bottom of the screen (e.g. "afraid", "angry") that best describes an affectively charged face at the top of the screen (Fig. 1B). This task requires a cognitive evaluation of the emotion displayed by faces viewed in the "perceive emotion" task. In the control condition, subjects matched one of two geometric shapes at the bottom of the screen to a target shape at the top of the screen (Fig. 1C).

The entire behavioral paradigm included nine experimental blocks and consisted of two "perceive emotion" blocks, two "label emotion" blocks and five control blocks. Each block lasted 32.5 s for a total scan length of 4:53 min. For each affect condition, 12 different images portraying an angry or fearful facial emotion were used (6 per block, 3 of each gender, all derived from a standard set of pictures of facial affect; Ekman and Friesen, 1976). "Perceive emotion" and "label emotion" blocks were interleaved with the five control blocks, and the order of task presentation was balanced equally in the manic and control subjects. During imaging, subjects responded by pressing one of two buttons with their right hand.

2.3. Image acquisition

MRI scans were obtained on a 3 T instrument (General Electric, Waukesha, Wisconsin, USA) with echo planar imaging capability (Advanced NMR Systems, Wilmington, Massachusetts, USA). An automated shim procedure was applied to maximize magnetic field homogeneity. A sagittal scout image (T_2 weighted) was obtained to

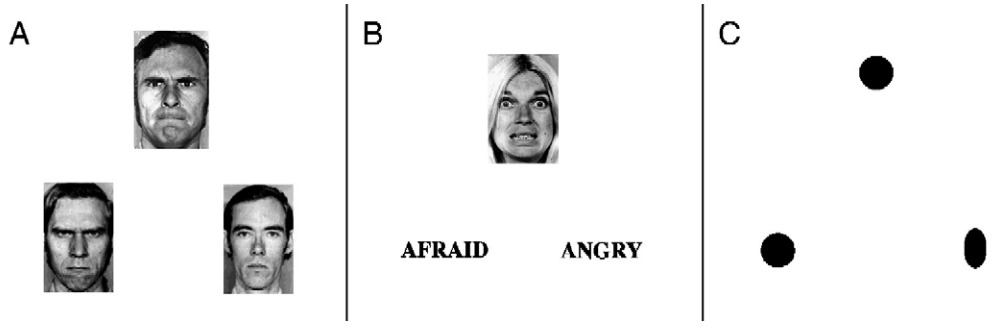

Fig. 1. Stimuli used in the (A) "perceive emotion", (B) "label emotion" and (C) control conditions.

identify locations for both structural and functional images. Slices for fMRI scanning covered 16 slices spanning the temporal lobes and upward (TR/TE/180 degree pulse offset = 2500/70/25 ms, 4 mm thick, 1 mm gap, matrix 64 × 64, FOV = 20 cm) using an asymmetrical spin echo acquisition sequence to reduce susceptibility artifact in the region of the amygdala and temporal poles. Co-planar EPI high-resolution structural images were obtained consisting of 26 slices (TR/TE = 4000/54 ms, 4 mm thick, 1 mm gap, matrix 128 × 128, FOV = 20 cm).

2.4. Behavioral data analysis

Response times and accuracy of performance of the task were recorded for patients and control subjects for the "perceive emotion", "label emotion" and control conditions. Differences between groups on each task were assessed with a mixed-effects analysis of variance model.

2.5. fMRI analysis

Functional images were examined closely for severe motion or spike artifacts. Single corrupted volumes containing spike artifacts and volume series or runs containing significant head motion of 2 voxels or greater were removed from further analysis. The total number of volumes removed from the patient group (mean 6.1 ± 3.7) did not differ significantly from the average number of volumes removed from the control group (mean 4.6 ± 4.7). Additional correction for head motion, spatial normalization, global scaling and realignment into atlas space was performed using Automated Image Registration (AIR) tools (Woods et al., 1998). Data were smoothed using a 6 mm full width at half maximum (FWHM) Gaussian kernel.

Statistical processing of image data was performed using the SPM2 software package (www.fil.ion.ucl.ac.uk/spm). Predetermined condition effects were calculated separately for each subject at each voxel using a *t* statistic, producing a statistical image for each contrast: "perceive emotion" vs. control, and "label emotion" vs. control. Contrast images generated for each subject were entered into a second-level random effects analysis to assess within- and between-group effects using one- and two-sample *t* tests, respectively. To test our a priori hypotheses, neuronal responses were identified at an uncorrected statistical threshold of $P < 0.005$. However, regions visible at this threshold for which we did not have a priori hypotheses (regions outside the VLPFC and amygdala) require replication in future studies to ensure their validity.

2.6. Functional connectivity analysis

Our primary interest in the present study was focused on assessing whether there was a negative functional connectivity (i.e. negative correlation of activations) that was present between the amygdala and VLPFC in healthy subjects during the psychological processes underlying the process of labeling of emotional stimuli vs. perception of these same stimuli, and whether the strength of this condition-specific negative functional connectivity was significantly decreased in bipolar patients experiencing symptoms of mania. We therefore performed a special type of functional connectivity analyses known as a psychophysiological interaction (PPI) analysis (Friston et al., 1997). PPI analyses measure the regionally specific activations of one brain area in terms of the interaction between input from another brain area and a psychological process (Friston et al., 1997). Such context-specific changes in functional connectivity are generally interpreted as contributory when the correlation in activity between two regions is positive (i.e. activity in area X triggers activation of area Y) or inhibitory when the correlation is negative (i.e. activity in area X suppresses activation in area Y) (Friston et al., 1997). However, it should be noted that because PPI is a correlational technique, the causal direction of functional connectivity cannot be conclusively determined.

Our PPI procedure was adapted from that of previous studies (Williams et al., 2006; Egner and Hirsch, 2004; Stephan et al., 2003; Orban et al., 2006) and used three regressors. The first regressor, known as the physiological variable, represented the time series of activity taken from the seed region, a single voxel of the amygdala. This voxel was determined separately for each subject, defined as the maximally activated voxel from the "perceive emotion" vs. the "match forms" condition, using a search region created from the combined amygdala activation cluster across patients and controls. The second regressor, known as the psychological variable, represented the condition type (coded by 1 for emotion labeling, −1 for emotion perception and 0 elsewhere). Coding of the condition type is used to determine the condition-specific changes in functional connectivity between two regions. The PPI variable represented the third regressor. This was computed as the cross-product of the first two regressors. This interaction term was created after the BOLD signal was deconvolved from the amygdala time series in order to represent the interaction at the neuronal level, by accounting for the hemodynamic time lag (Gitelman et al., 2003).

Table 1
Mean behavioral scores

	Group	Median	Mean	S.D.	*P*
"Perceive emotion"					
RT	Controls	2.24	2.22	0.59	0.17
	Patients	2.43	2.31	0.34	
Accuracy	Controls		0.76	0.15	0.93
	Patients		0.89	0.09	
"Label emotion"					
RT	Controls	2.50	2.52	0.52	0.14
	Patients	2.70	2.52	0.75	
Accuracy	Controls		0.77	0.14	0.28
	Patients		0.82	0.12	

RT, response time (s); S.D., standard deviation; Accuracy, proportion of correct responses.

To determine which areas of the brain contained activity that was predicted by the PPI variable, a new linear model was built for each subject that incorporated this interaction term. Applying a *t*-contrast that was −1 for the PPI regressor and 0 elsewhere produced subject-specific statistical images that revealed brain voxels having a significant negative regression slope ($P<0.05$) with activity in the left amygdala during the emotion labeling condition vs. the emotion perception condition. Subject-specific statistical PPI images were taken to a second-level random effects analysis to evaluate within- and between-group differences using one- and two-sample *t* tests, respectively. Given our strong a priori hypotheses, PPIs between the amygdala seed region and BA47 regions were identified using an uncorrected statistical threshold of $P<0.005$. Other regions for which we did not have an a priori hypothesis, such as anterior cingulate to amygdala were also identified at this threshold, but require further study to establish their validity.

Associations between VLPFC–amygdala negative functional connectivity and clinical variables (illness duration, number of prior manias and YMRS scores) were assessed within manic subjects via correlation analyses in SPM. Specifically, voxels of the VLPFC which had a connectivity strength with left amygdala that was significantly correlated with these variables were identified using a search region defined by the PPI group map of healthy subjects.

3. Results

3.1. Behavioral responses

No significant between-group differences were found for response times or accuracy of responses during performance of the "label emotion" ($P=0.14$ and $P=0.28$, respectively) or "perceive emotion" tasks ($P=0.17$ and $P=0.93$, respectively, see Table 1).

Table 2
Regions of significant activation between groups

	Talairach coordinates (*x*, *y*, *z*)	Cluster size (no. of voxels)	*Z* score
Patients vs. controls			
"Perceive emotion" vs. control			
Amygdala	−18, −4, −12	7	2.72
	−18, −28, −16	4	
Thalamus	−4, −28, 6	18	2.98
Anterior cingulate (BA24/32)	−16, 42, 2	5	3.19
	8, 24, 24	4	3.11
"Label emotion" vs. control			
Amygdala	−18, −12, −12	10	3.07
	−26, −6, −8	6	2.83
Thalamus	−4, −12, 10	19	3.44
Middle/inferior frontal gyrus (BA46)	−32, 30, 14	15	3.28
Controls vs. patients			
"Perceive emotion" vs. control			
Inferior frontal gyrus (BA47)	−34, 28, −8	126	4.33
	34, 34, −4	19	3.18
	36, 24, −4	9	2.97
Anterior cingulate (BA32)	−14, 34, 18	15	2.94
"Label emotion" vs. control			
Inferior frontal gyrus (BA47)	−34, 26, −8	9	3.03
	36, 30, −10	3	2.91
	44, 24, −8	2	2.75
Ventral anterior cingulate (BA24)	−6, 34, 4	179	3.84
Middle frontal gyrus (BA9)	−46, 26, 28	9	4.13

Coordinates represent voxels in each region with the most significant magnitude and spatial extent, identified at an uncorrected threshold of $P<0.005$.

3.2. BOLD fMRI responses

The aim of this study was to examine group differences in the negative functional connectivity between prefrontal regions and the amygdala, and as such, both the within- and between-group connectivity findings are presented. For clarity, we present the results of between-group comparisons for the two task contrasts ("perceive emotion" vs. control and "label emotion" vs. control). A portion of the results from the within-group analysis for the "perceive emotion" vs. control conditions has been published previously (Altshuler et al., 2005a), and additional details of the within-group analysis results are available from the authors upon request.

3.3. Between-group contrasts

Table 2 summarizes the significant between-group differences in BOLD fMRI activations from the "perceive emotion" vs. control and "label emotion" vs. control contrasts. Patients, compared to healthy subjects, yielded significantly greater activation of the left amygdala and reduced activation of the VLPFC (BA47) in both contrasts (Fig. 2). In the "label emotion" vs. control contrast, patients also demonstrated increased activation in the superior portion of the inferior frontal gyrus (BA46) and decreased activation of the ventral ACC (BA24) compared to healthy subjects.

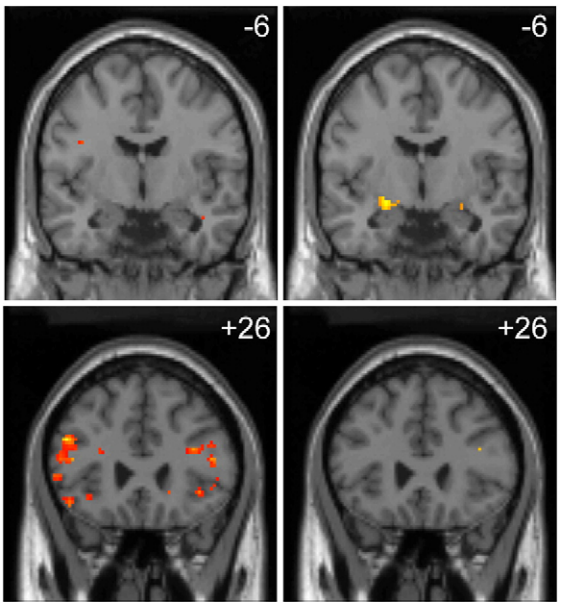

Fig. 2. BOLD signal responses in healthy subjects (left column) and bipolar-manic subjects (right column) during the "label emotion" vs. control conditions. Activation of the VLPFC appears absent in bipolar subjects, and is accompanied by increased response of the left amygdala. Shown at $P<0.005$, uncorrected, extent threshold $k=5$ voxels. Image left corresponds to subject left.

Table 3
Regions found to be negatively functionally connected with the response of the left amygdala: within- and between-group results from PPI analyses

	Talairach coordinates (x, y, z)	Cluster size (no. of voxels)	Z score
Controls			
Ventral PFC	−42, 28, −8	32	3.48
(BA47)	46, 32, 0	11	3.40
Anterior cingulate (BA32)	12, 24, 30	12	3.43
Patients			
Anterior cingulate	−4, 38, 18	15	3.18
(BA32/BA24)	−12, 8, 30	13	3.13
Middle frontal gyrus (BA10)	36, 48, 8	12	2.97
Patients vs. controls			
Anterior cingulate gyrus (BA32/BA24)	−4, 38, 16	9	2.89
Middle frontal gyrus (BA10)	36, 48, 4	12	3.02
Controls vs. patients			
Ventral PFC	46, 32, −2	16	3.12
(BA47)	−42, 26, −4	23	3.71

Coordinates represent voxels in each region with the most significant magnitude and spatial extent, identified at an uncorrected threshold of $P<0.005$.

3.3.1. PPI results

PPI results are summarized in Table 3. Healthy subjects demonstrated negative functional connectivity between the response of the left amygdala and that of bilateral VLPFC (BA47) and right ACC (BA32), whereas patients demonstrated negative functional connectivity between the response of the left amygdala and left ACC (BA32/24) and right middle frontal gyrus (BA10). These differences were further reflected in direct group comparisons (Fig. 3, Table 3), with bilateral VLPFC–left amygdala negative functional connectivity greater in healthy subjects, and left ACC– and right BA10–left amygdala negative functional connectivity significantly greater in patients. Because our hypothesis was limited to one pathway, i.e. VLPFC to amygdala, whole brain correction was not required to validly interpret the significance of this finding; however, other effects (ACC to amygdala, etc.) require further study.

3.4. Relationship of BOLD fMRI responses with illness duration, symptom severity and prior manias

Correlation analyses showed a significant inverse relationship between YMRS scores and left VLPFC–

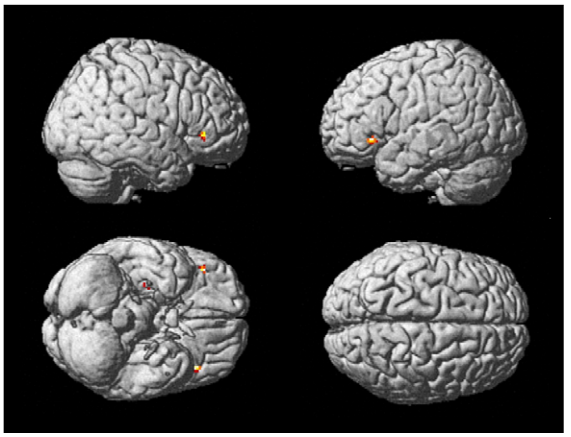

Fig. 3. Direct group comparison (controls vs. patients) of the negative functional connectivity with left amygdala that occurs during the "label emotion" condition. Shown at $P<0.005$, uncorrected, extent threshold $k=15$ voxels.

left amygdala negative functional connectivity ($k=1$ voxel; x, y, z Talairach coordinates $=-40$, 24, -6, $r=-0.84$), the number of prior manic episodes and right VLPFC–left amygdala negative functional connectivity ($k=3$ voxels; x, y, z Talairach coordinates $=44$, 34, -2, $r=-0.89$), and between illness duration and right VLPFC–left amygdala negative functional connectivity ($k=1$ voxel; x, y, z Talairach coordinates $=46$, 34, -2, $r=-0.84$). No significant correlations were found for age.

4. Discussion

Consistent with previous reports (Hariri et al., 2000, 2003; Lieberman et al., 2005), we found negative functional connectivity between the amygdala and VLPFC in healthy subjects during the cognitive evaluation (labeling) of affective stimuli. That is, when normal control subjects evaluate a facial expression by identifying an emotion displayed by a face and assign a verbal descriptor to that emotion, a neural network becomes activated in which the VLPFC appears to exert an inhibitory effect on amygdala. This negative functional connectivity pattern was significantly reduced in manic patients, who instead demonstrated an increased BOLD response in the amygdala and a decreased BOLD response in VLPFC during the cognitive evaluation task compared to control subjects. Only three known studies of bipolar mania have previously targeted prefrontal function using behavioral inhibition tasks in conjunction with fMRI and of these, all have reported decreased activation of the VLPFC in comparison with healthy subjects (Blumberg et al., 2003; Elliott et al., 2004; Altshuler et al., 2005b). Our replication of decreased function in VLPFC using a different task paradigm underscores the role of this frontal region in bipolar mania and extends previous findings by linking this functional deficit with an increased responsivity of the left amygdala.

Disruptions of frontal–limbic networks are beginning to emerge in other studies of emotional disorders, including schizophrenia (Meyer-Lindenberg et al., 2001; Das et al., 2006), borderline personality disorder (New et al., 2007) and unipolar depression (Pezawas et al., 2005). To our knowledge, this is the first study to examine the functional connectivity of mood regulatory networks in bipolar disorder. A disruption of the fronto-limbic circuitry in bipolar mania may have special clinical relevance; an inverse correlation was found between the current episode's severity of mania and the left VLPFC–left amygdala functional connectivity. An inverse correlation was also found between the number of prior manias and the strength of right VLPFC–left amygdala connectivity such that a greater number of prior manias were associated with a more weakened connectivity between fronto-limbic centers. Finally, a significant negative correlation was found between illness duration and right VLPFC–left amygdala functional connectivity. Future studies that examine whether each manic episode cumulatively contributes to a permanent alteration in right sided fronto-limbic emotion modulatory circuits would be of interest, as would studies that examine whether medication significantly alters fronto-limbic connectivity.

In contrast to findings from previous studies (Getz et al., 2003; Lembke and Ketter, 2002) bipolar subjects in our sample did not perform significantly worse on the behavioral measures of emotion perception or emotion labeling compared to healthy subjects. We believe this to be because unlike previous studies, our facial emotion task was not difficult. That is, the present study was conducted not to ascertain whether there are subtle perceptual differences between groups, but to show how strong emotional faces might differentially activate the amygdala. Thus, deficits in the recognition of facial emotion if present, were not discernible in our bipolar sample using the affective labeling task employed here. This is an area worthy of further exploration however, as pathological hyperactivity of the amygdala may be critical in how affective information is perceived. Both studies of healthy individuals and unipolar-depressed subjects have shown that the degree of amygdala hyperactivity is directly correlated with negative biases in the perception of facial emotion (Cooney et al., 2006; Dannlowski et al., 2007). Future paradigms that

therefore employ more difficult facial recognition paradigms may allow for evaluation of correlation between deficits in facial recognition in bipolar disorder and amygdala activation.

Our finding of increased amygdala response in manic patients is not unique to the state of mania. Hyperactivity of this structure has been reported in studies of euthymic patients both in cases where the valence of perceived emotional facial expressions was positive (Lawrence et al., 2004) and negative (Yurgelun-Todd et al., 2000; Lawrence et al., 2004). One recent study showed that a heightened response of the amygdala occurs even to neutral faces in a sample bipolar-euthymic youths (Rich et al., 2006). Taken in context with other studies showing that a reduction in ventral prefrontal function (Malhi et al., 2005; Strakowski et al., 2005) as well as a heightened emotional reactivity (Lovejoy and Steuerwald, 1995) persists beyond manic and depressive states, it seems likely that a weakening of frontal regulation of limbic centers may indeed be a trait related abnormality that could contribute to vulnerabilities in mood.

Increased response of the amygdala in bipolar mania provides one possible explanation for the enlargement of this structure reported by our group (Altshuler et al., 1998), and others (Brambilla et al., 2003; Strakowski et al., 1999), although not all groups have observed this (see Blumberg et al., 2003). A reduction in modulatory/inhibitory VLPFC inputs could lead to increases in amygdala firing, and trigger dendritic arborization in this subcortical region (Vyas et al., 2002). Frontal dysfunction in turn may be caused by reductions in frontal lobe gray matter (Frangou, 2005; Lopez-Larson et al., 2002; Lyoo et al., 2004). Future studies are required however to further assess the relationship between brain structure and function, and the impact of mood state.

While manic patients in our sample demonstrated a robust increase in activation of the amygdala relative to healthy subjects in both the "perceive emotion" and "label emotion" conditions, previous findings regarding amygdala function in bipolar mania have been inconsistent (Lennox et al., 2004; Chen et al., 2006). It may be possible that differences in ours and others' findings are due to variations in the clinical profiles of the manic subjects included in each study, however we suspect that differences in activation are more likely the result of variations in activation paradigms (Critchley et al., 2000). Chen et al. (2006) who scanned bipolar-manic, bipolar-depressed and healthy subjects found that subjects with mania elicited a greater activation of the amygdala compared to healthy subjects when they rated the intensity of a color shaded over an emotional face, but these same patients elicited a reduced activation of the amygdala compared to healthy subjects when they rated the emotional intensity of that face. Amygdala activations during mania are therefore likely strongly influenced both by the type of activation task employed and/or the by the level of attentional engagement during the processing of emotional stimuli (Chen et al., 2006). Future studies that further delineate the impact of task design on amygdala functioning and their interaction with mood state are needed.

Although greater VLPFC activation and negative functional connectivity with the amygdala in healthy subjects was predicted, its apparent bilaterality was surprising, given previous reports of right sided frontal connectivity (Hariri et al., 2000, 2003; Ochsner et al., 2004; Lieberman et al., 2005). Lack of power may be to blame, as the current study included only nine subjects in each group, whereas studies showing right sided prefrontal–amygdala connectivity have included between 16 and 24 subjects. Functional neuroimaging studies of manic subjects however are commonly based on small samples; previous reports have included as few as 5 (Blumberg et al., 1999), 6 (Rubinsztein et al., 2001), 8 (Elliott et al., 2004) and 11 manic subjects (Blumberg et al., 2003, 2005). The logistical problem of having a manic patient remain at rest for a period of time no doubt accounts for the small numbers. In the current study, more severely manic patients were excluded from further analysis due to excessive motion.

Group differences in VLPFC–amygdala coupling were the primary focus of this study, but several other regions were differentially activated and found to be functionally connected with the amygdala between groups. However, because these regions were not part of our a priori hypotheses and as such, did not survive a whole brain corrected threshold of $P<0.05$, an interpretation of their role in emotion processing is not included here. Future studies that observe these regions to be differentially activated between groups, perhaps in a study employing a larger number of subjects, would help to ensure the validity of these findings.

To our knowledge, this is the first study to evaluate the functional connectivity between frontal and limbic regions in bipolar disorder. Our findings should be considered in the light of several limitations. First, PPI is only able to account for the functional connectivity between a seed region and regions to which activity in the seed region is directly coupled. Therefore, future studies that utilize more complex connectivity analyses, such as dynamic causal modeling (Stephan et al., 2003) or structural equation modeling (Horwitz et al., 1999) are needed to be able to account for the effect of

additional brain regions in emotion regulatory circuits on amygdala activity.

Second, many of our subjects were on antimanic medications at the time of scanning, and the impact of these medications on blood flow is unknown. Medication effects may contribute to the heightened left amygdala response in patients, but previous studies of mood disordered subjects observed both pre- and post-medication intervention have shown that pharmacological treatment either did not detectably change blood flow (Oliver and Dormehl, 1998; Theodore, 2000) or reduced it (Leiderman et al., 1991; Gaillard et al., 1996; Blumberg et al., 2005).

Third, reduced activation in VLPFC in manic subjects may be the result of their attending less to the task; however, accuracy and response times suggest that all subjects were attending to the task and make this explanation of our findings unlikely. Moreover, activity in the fusiform gyrus, a region which consistently responds to viewing of faces and which can be directly modulated by attention (Pessoa et al., 2002), was not significantly different between groups.

Fourth, more severely manic patients had to be removed from the current analysis due to motion thus limiting the range of YMRS scores in our sample. This, combined with the fact that most of our subjects were on medication, constrains the ability to draw clear conclusions from this study. However, even within the narrow YMRS range, correlations were seen between mania severity and negative connectivity.

Finally, PPI does not resolve causality, therefore it may be argued that the amygdala suppresses VLPFC output. We consider this possibility to be unlikely however, since more unilateral projections travel from VLPFC to the amygdala than vice versa (Cavada et al., 2000) and at least a portion of these efferents are directly inhibitory (Amaral et al., 1992; Cavada et al., 2000; Ghashghaei and Barbas, 2002). Additionally, lesion or inactivation of the ventral PFC has been found to additionally facilitate affective behaviors, whereas stimulation of the PFC suppresses these behaviors (al Maskati and Zbrozyna, 1989; Dias et al., 1996).

In summary, consistent with previous reports we found negative (i.e. modulatory) functional connectivity between the left amygdala and bilateral VLPFC in healthy subjects during the cognitive evaluation of affective stimuli. This connectivity pattern was significantly decreased in bipolar-manic patients, who additionally demonstrated increases in the response of the amygdala and decreases in the response of the VLPFC. Our finding of decreased functioning in the VLPFC using a task paradigm different from previous studies underscores a role for dysfunction in ventrolateral prefrontal brain regions in bipolar mania and extends these findings by linking this deficit with increased responsivity of the amygdala.

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