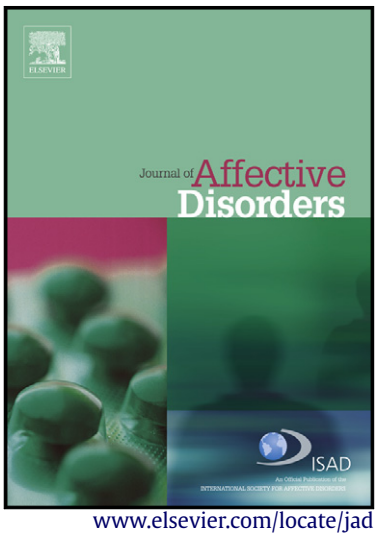
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DIFFERENTIAL ABNORMALITIES OF FUNCTIONAL CONNECTIVITY OF THE AMYGDALA AND HIPPOCAMPUS IN UNIPOLAR AND BIPOLAR AFFECTIVE DISORDERS

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ABSTRACT

Objective: The amygdala and hippocampus – two structures intimately associated with mood and cognition – have been reported to exhibit altered neural activity or volume in affective disorders. We hypothesized the amygdala and hippocampus would show altered and differential patterns of connectivity in patients with bipolar (BPs) and unipolar (UPs) disorder compared to healthy volunteers.

Method: Thirty BPs, 34 UPs, and 66 healthy volunteers were imaged using F-18-fluorodeoxyglucose and positron emission tomography while performing an auditory continuous performance task (CPT). Normalized mean activity of the amygdala and hippocampus was correlated with the rest of the brain.

Results: In BPs, the amygdalae displayed exaggerated positive metabolic correlations with prefrontal and ventral striatal areas, while the hippocampus showed a paucity of normal inter-relations compared to controls. In contrast, in UPs the amygdala was significantly negatively correlated with prefrontal and anterior cingulate cortex, while the hippocampus was significantly more positively correlated to these same prefrontal areas.

Conclusions: During a simple cognitive task, the functional connectivity of the amygdala and hippocampus, regions usually associated with emotion and memory regulation, was substantially different in affective illness compared to healthy controls whether or not there were baseline abnormalities in these areas. These striking differences in functional connectivity of amygdala and hippocampus should be further explored in ill and well states and using more specific emotion and cognitive evocative tasks.

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Introduction

The amygdala and hippocampus are considered critical structures contributing to the dysregulation of emotional and cognitive functions, respectively, in bipolar (BP) and unipolar (UP) affective disorders. Each structure has been extensively studied and linked mechanistically to the symptomatic aspects of BP and UP disorders (Price and Drevets, 2012). Thus, the careful dissection of type of dysfunction or dysregulation of these structures would add considerably to the understanding of the pathophysiological processes underlying these two disorders. However, functional and structural neuroimaging findings supporting this belief have been variable (see reviews Strakowski et al., 2005; Drevets and et al., 2008; Cerullo et al, 2009), so that the precise nature of these abnormalities and their consequences remain to be established. Furthermore, the emotional and cognitive deficits patients experience could be related not only to altered levels of activity within each region, but also to abnormal interregional relationships of these two critical regions with other brain areas.

Affective instability typifies bipolar illness where mood swings from depression to mania can cycle within months, days or even hours (Goldberg et al., 2009). Unipolar illness, on the other hand, is mainly characterized by low mood, anhedonia, and a host of vegetative disturbances, and is distinguished from bipolar disorder by the lack of manic episodes. Amygdalar and hippocampal dysfunction is thought to play a major role in these symptoms, often figuring predominately in models of affective disorders (Krishnan and Nestler, 2011; Price and Drevets, 2010; Strakowski et al., 2005).

Evidence of amygdala dysfunction in bipolar illness comes from neuroimaging studies. For example, increased baseline activity can occur in the depressed state (Drevets 2002; Ketter et al., 2001) and in mania (Altshuler et al., 2005) compared to controls, and may normalize with euthymia (Ketter et al., 2001). When bringing the amygdala on-line

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with emotion tasks, increased activity has been observed in children (Chang, 2005; Leibenluft and Rich, 2008) and adults (Yurgelin-Todd et al., 2000) with bipolar disorder. It is unclear how these differences in activity relate to abnormalities in amygdalar volumes in bipolar illness that appear to vary across the life span (see review, Konarski et al., 2008; Sheline, 2003, as many investigators may not apply volume correction.

Similarly in unipolar depression, increased amygalar activity has been reported at rest (Drevets, 2000, 2003; Nofzinger, et al., 1999; Siegle et al., 2002), during REM sleep (Ho et al., 1996), with sadness induction (Surguladze et al., 2005), and when viewing fearful or smiling faces (Sheline et al., 2001), or aversive (Heinz, et al., 2005) or negative (Anand et al., 2005) pictures. However decreased amygdala activity was also shown at rest (Anand et al., 2005) or during continuous performance task (CPT; Kimbrell et al., 2002) in UPs compared to controls.

Pessoa (2008) argues that emotion is not solely parceled into the limbic system and affective regions, but also involves functions in the cognitive domain, specifically to monitor potential salient stimuli. Thus, in addition to the emotion dysregulation, impairment in the cognitive domain also occurs in mood disorders including abnormalities of verbal and spatial memory, facial emotion recognition, and the inability to concentrate and maintain attention (Sheline, 2003). Attention deficits may also be a trait marker of mood disorders (Najt, et al., 2005).

Consistent with this view, amygdalar activity is elevated during sustained attention in bipolar mania (Fleck et al., 2010). Moreover, normal amygdala activity is suppressed during cognitive engagement (Pessoa, 2005). Attention and executive functioning, which depend on these memory skills, may be compromised with greater illness severity (Altshuler et al., 2008a; McClintock et al., 2010), however, deficits persist in euthymia (Bearden et al., 2010). Yet, hippocampal activity in BPs (Bauer et al., 2005; Ketter et al.,

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2001; Chen et al., 2005) is not consistently abnormal; metabolic activity has been reported as increased (Bauer et al., 2005; Ketter et al., 2001, no change (Drevets et al., 2002), and may differ across mood states with higher activity in mania compared to depression (Chen et al., 2005). In contrast, hippocampal activity in UPs is often reported as abnormal, but there are discrepancies in the direction of change compared to controls.

While there are no consistent abnormalities in hippocampal volumes in biplar illness (Sheline, 2003), a large and highly consistent literature indicates that UPs have reduced hippocampal volume (Sheline et al., 1996; Sheline, 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004). Reports of amygdala volumes are variable (Sheline et al., 1998; Lange et al., 2004; Hastings et al., 2004; Rosso et al., 2005). Several investigators describe the left amygdala and right hippocampus were larger than their contralateral homologous structures (Bremner et al., 2000; Frodl et al., 2002, 2004; Mervaala et al., 2000). Thus, laterality may be important factor in unipolar illness.

These inconsistencies in discrete regional amygdalar and hippocampal structure and function in mood disorders suggest that other factors may contribute to these discrepancies. Questions remain as to how the functional significance of amygdala and hippocampal activity and volume changes relate to the other common findings in the affective disorders, such as decreased activity in prefrontal lobe areas (see reviews, Drevets et al., 2008; Strakowski et al., 2005).

One possible explanation is that the absolute or relative regional activity in the amygdala and hippocampus is only one aspect of dysfunction, and the functional relationships between these two key limbic regions and the regions to which they connect may explain the dysfunction these patients experience. For instance, initial studies reported increased functional connectivity of the amygdala in BPs and UPs compared to

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controls during emotive tasks (Irwin et al., 2004; Matthews et al., 2008; Yoshimura et al., 2010; Versace et al., 2010) and decreased during rest (Anand et al., 2005, 2009).

Using the methods of Horowitz et al., (1990; 1991), we sought to examine the functional relationships of the amygdala and hippocampus during a simple cognitive task, where performance differences could be eliminated, and at the same time elucidate mechanisms of attention in a low-stress task. This paradigm may provide an ecologically sound paradigm where the simple cognitive activity leaves the opportunity of emotion to intrude in these emotionally labile patients, possibly mimicking everyday experiences.

We previously found that BPs displayed a general pattern of increased functional connectivity of the dorsolateral prefrontal cortex (DLPFC), insula, thalamus, inferior parietal cortex and cerebellum with many brain area compared to UPs and controls (Benson et al., 2008). In this study, we focused on the amygdala and hippocampus and postulated they would also exhibit a pattern of metabolic hyper-connectivity in BPs and hypo-connectivity in UPs similar to that seen in cortical and thalamic regions (Benson, et al., 2008; Willis et al., 2008). In addition, because of reports in UPs of increased left amygdalar and reduced right hippocampal volumes (Mervaala et al., 2000), we hypothesized that in UPs the connectivity would differ as a function of laterality.

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Methods and Materials

Subjects

The subjects involved in this study, as detailed previously in (Benson et al., 2008; Willis et al., 2008), are described in Table 1. Thirty treatment-refractory inpatients with bipolar disorder (18 men (M) and 12 women (F); mean age: 36.4 ± 10.6 [mean ± SD], days med-free: 29.8 ± 38.3; Ketter et al., 2001) and thirty-four patients with unipolar depression, including treatment-refractory inpatients (Kimbrell et al., 2002) as well as outpatients with

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less challenging illness (Little et al., 1996; 2005) (18 M and 21 F; mean age: 43.0 ± 13.3; days med-free 68.9 ± 95.1) were imaged medication-free. The mean age of the two patients groups slightly differed (t=2.20, p=.03, df=62), which was controlled statistically (see below). All patients were assessed with ratings of depression, mania, and anxiety on the day of the scan (Hamilton, 1960; Beck, et al., 1961; Young et al., 1978; Spielberger et al., 1983

Clinical characteristics, including illness severity, time-course measures, comorbidity, and family history were collected, in some instances on a subset of the patients (BP, n=26; UP, n=30). The two patient groups had comparable levels of illness severity as assessed by number of ratings of depression, anxiety, failed medication trials, and refractory indices. One discrepancy, was the increased number of prior depressive episodes in BPs (t=-3.64, p=.001, df=54). However, weeks-ill was not significantly different, suggesting shorter episodes accompanied the greater cyclicity in BPs. Comorbid diagnoses included anxiety disorders (BP: n=4, UP: n=5) and substance abuse (BP: n=4, UP: n=7). Family history broke down as follows: 1) in BPs, bipolar illness (n=11), depression (n=6), anxiety (n=4), and substance abuse (n=3); and 2) in UPs, bipolar illness (n=2), depression (n=13), one with anxiety (n=1), and substance abuse (n=2).

Each patient group was compared to 66 healthy controls, (Willis et al., 2008; (38 M and 28 F; age: 39.3 ± 11.2), who were carefully screened for the absence of major Axis I or Axis II psychiatric disorders (First, et al., 2001). All participants gave written informed consent for the studies and procedures involved as approved by the National Institute of Mental Health (NIMH) Institutional Review Board.

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FDG PET Scan Procedure

All subjects were imaged with a Scanditronix PC1024-7B scanner (Uppsala, Sweden) with seven tomographic slices, in-plane full width half-maximum (FWHM) resolution of 6.5 mm, and slice thickness of 10 mm at the center of the field of view. After obtaining a transmission scan, the subjects' eyes were covered and headphones placed over their ears, and fluorine-18 deoxyglucose (FDG) was administered intravenously (4-5 mCi). During the FDG uptake period, all subjects performed an auditory continuous performance task (CPT) for 30 minutes (Cohen et al., 1988). In this relatively simple cognitive task, the subject pressed a button upon identification of a medium-level tone of three tones. Then, four emission scans were collected with seven slices were acquired, resulting in 28 planes spaced 3.5 mm apart, starting 8.5 mm above the canthomeatal line. Images of regional cerebral metabolism were obtained from the emission scans and time-radioactivity data using the model of Sokoloff (1981).

Image and Statistical Analyses

Image processing and analysis was previously outlined (Willis, et al., 2008). Image processing and analysis were performed on UNIX workstations (Sun Microsystems Inc.) using Statistical Parametric Mapping (SPM) software. ANALYZE (Mayo Foundation, Rochester MN) and NIH- and NIMH-developed software was also used in the image processing, analysis and presentation. To be compatible with our studies regarding characterization of baseline metabolism (Ketter et al., 2001; Kimbrell et al., 2002), identical image processing methods were used. Scans were visually inspected for artifacts and stereotactically normalized using a histogram equalization-based non-linear transformation as described previously (Ketter et al., 1999; Willis et al., 2002) into a standard space corresponding to the human brain atlas of Talairach and Tournoux (1988). These images

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were then smoothed with a Gaussian low-pass filter of 10 mm in-plane and 6 mm axial FWHM (in order to minimize noise and improve between-subject spatial alignment) for a final resolution of approximately 12 mm isotropically. Gray-matter estimation was achieved by first estimating the background noise threshold (i.e., non-brain) as one-eighth of the mean of the entire image space, and voxels exceeding 60% of the whole-brain mean were then assumed to be gray matter. Global metabolic rate for glucose (gCMRglc) was then calculated as the mean of these voxels.

Bilateral amygdalar and hippocampal regions of interest (ROIs) were defined from the Talairach atlas (Talairach and Tournoux, 1988) and their neuroanatomical location was verified by overlaying these templates on the magnetic resonance imaging (MRI) template supplied with SPM. The mean value for amygdala and hippocampus metabolism was computed separately for the left and right ROI, then proportionally normalized, age adjusted using the age regression model of Willis et al (2002; 2008). In each group, mean ROI values were correlated with activity in the rest of the brain activity using GLM, where amygdala activity was the regressor of interest and mood ratings (28-item Hamilton Depression Rating Scale [HDRS]; Hamilton 1960) were covaried in order to remove any potential metabolism-mood confound in the investigation of connectivity. Differences between the correlative relationships in patients from healthy volunteers were also assessed in SPM. Significant clusters surpassed the multiple comparison method of cluster analysis (Z maxima of $p<.001$ and a cluster probability threshold of $p = .05$; Friston et al., 1994). The Talairach and Tournoux atlas (1988) was used to identify brain regions with significant findings, including Brodmann's areas when possible.

To address laterality, we compared the number of either positive or negative relationships as a function of laterality within and between each group. The number of

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voxels with significant ($p<.01$) positive and negative correlations for a given region of interest was cross-tabulated by diagnosis subdivided by laterality (Figure 4; table format available online). We chose to report Φ (Rosenthal, 1991), where $\Phi = \sqrt{(\chi^2 / N)}$ are interpretable as an effect size. Positive Φ values indicate a greater number of significant positive correlations, and negative Φ values more negative correlations. In order to correct for Type II errors, only values of $\Phi \pm .30$ or greater, i.e., a medium effect size (Cohen and Cohen, 1983), were considered.

Results

The mean globally normalized metabolic activity of the amygdala was significantly different among groups. This was due to greater amygdala activity in BPs compared to either UPs or controls (C). This difference existed in both the left amygdala (BP: 72.0 ± 6.0; UP: 68.5 ± 5.0; C: 69.8 ± 5.2; $F(2,129)=3.60$, $p=.03$) and the right amygdala (BP: 73.4 ± 5.0; UP: 69.5 ± 4.0; C: 69.7 ± 5.2; $F(2,129)=7.20$, $p=0.001$). Activity did not differ significantly activity in the UPs compared to controls.

There were no significant differences in mean metabolic activity of the hippocampus on either the left (BP: 68.0 ± 4.7; UP: 69.1 ± 5.9; C: 67.8 ± 5.3; $F[2,129]=0.67$, p=ns) or the right (BP: 70.5 ± 5.6; UP: 69.6 ± 4.8; C: 68.3 ± 4.3; $F[2,129]=2.34$, $p=.10$) across the groups.

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Connectivity of the Amygdala

Metabolism in the left and right amygdalae of controls (Willis et al., 2008) was significantly positively correlated with both ipsilateral and contralateral amygdala and surrounding ventral striatum (Figure 1; top, middle, and lower panels, slices -16 through 0). The amygdala and medial orbitofrontal cortex were inversely related in controls.

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Metabolism in the amygdalae in BPs was significantly more positively correlated with both the ipsilateral and contralateral amygdala and the surrounding ventral striatum (Figure 1; top, middle, and lower panels, slices -16 through 0. In addition, instead of normal negative correlation of the amygdala with the orbitofrontal cortex in controls, in BPs the correlations of amygdala activity with the orbitofrontal cortex were reversed and positive. When compared to controls, BPs showed significantly greater magnitude of positive relationships of the amygdala with the orbitofrontal cortex, right hippocampus, left temporal cortex, and right cuneus/fusiform cortex (Table 2 and Supplementary Figure 1). There were no relationships significantly more negative than in controls.

In UPs, each amygdala was not as highly positively correlated with the contralateral amygdala and surrounding regions, as was seen in BPs or controls (Figure 1, lower panel, slices -16 and -8). Both amygdalae negatively correlated with left DLPFC (BA 45/46), but only the right amygdala unilaterally negatively correlated with the right DLPFC (BA 46). When the magnitude of these relationships was directly compared to controls (Table 2 and Supplementary Figure 2), metabolism in both amygdalae was significantly more negatively correlated with left DLPFC and anterior cingulate in UPs than controls.

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Hippocampal Relationships

The normal connectivity pattern of the hippocampus (Figure 2; top panel) includes a predominance of negative correlations with cortical regions and positive relationships with subcortical areas, similar to that seen in the thalamus (Willis et al., 2008). In patients with bipolar illness, the hippocampus showed fewer significant correlations of either valence with other brain areas (Figure 2; middle panel). Each hippocampus positively correlated primarily with ipsilateral hippocampus and the surrounding parahippocampal gyrus, and negatively with right DLPFC. Compared to controls, the left hippocampus in bipolar

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patients showed significantly greater positive correlations with posterior regions of the brain, including the mid-temporal and occipital cortex as well as the cerebellum, and more negative correlations with bilateral DLPFC (Table 2 and Supplementary Figure 3). The right hippocampus in BPs was significantly less negatively correlated than in healthy controls with anterior regions, bilateral medial frontal and anterior temporal cortex.

In UPs, relationships of the hippocampi with the rest of the brain had a generally similar pattern to the controls, although there were altered relationships to prefrontal areas. The left hippocampus in UPs (Figure 2; bottom panel) had significant negative correlations with left subgenual anterior cingulate and the inferior, ventrolateral, and dorsolateral prefrontal cortices that were not observed in controls (Figure 2; top panel), and each of which had significantly greater negative values than controls (Table 2 and Supplementary Figure 4). The right hippocampus, however, lacked the normal significant negative correlations with mid-frontal and anterior cingulate cortex; instead these regions were significantly less negatively correlated in the unipolars than controls.

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Laterality

When comparing the number of correlations with a magnitude significantly different from controls, in UPs there was an opposite effect of laterality with greater connectivity of the left (322/158) and lesser of the right amygdala (43/821), resulting in a robust laterality effect ([L/R]; $\Phi = -.67$).

The opposite occurred in the hippocampus of the UPs; the valence ratios were exclusively negative on the left (0/245) and relatively more positive on the right (170/37), yielding a large laterality effect size ([L/R]; $\Phi = .85$). In contrast, in BPs, there were no significant laterality differences in amygdalar or hippocampal relationships.

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Discussion

Overview

During a simple attention task, we found marked differences in functional connectivity of the amygdala and hippocampus in both bipolar and unipolar illness compared to controls that also robustly distinguished the two disorders. These differences are summarized in Figure 3. As postulated, patients with bipolar illness demonstrated a pattern of enhanced positive connectivity of the amygdala with other brain areas similar to that seen in cortical, thalamic, and cerebellar areas (Benson et al., 2008). However, contrary to our prediction, the hippocampus showed a relative paucity of interregional relationships. A double dissociation was apparent as patients with unipolar illness the showed the opposite pattern of a relative paucity of interregional correlations of the amygdala, and conversely the hippocampus had a more normal pattern with additional relationships to prefrontal areas not seen with controls. The presence of this double-dissociation in patterns of amygdala versus hippocampus interregional connectivity in bipolars compared to unipolars further suggests that many of the methodological limitations considered below are not likely to account for the findings of this study.

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Functional Connectivity of the Amygdala

The functional connectivity of the amygdalae in bipolars differed from controls in that the relative balance of correlations of the amygdalae with other brain regions was skewed to almost exclusive positive relationships. This was a function of two general patterns: 1) there were fewer of the normal inverse correlations with prefrontal regions; and 2) metabolism was significantly more positively correlated with posterior regions, left fusiform / middle temporal gyrus, precuneus, and cerebellum.

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Thus, in addition to the common theme of positive hyper-connectivity previously seen of the dorsolateral, insular and parietal cortex and and thalamus with other brain regions in bipolars (Benson et al., 2008), the amygdalae also shared enhanced functional connectivity with MPFC and posterior brain regions including the cerebellum. These findings during a CPT task are partially consistent with the work of Foland et al., (2008) showing that during mood elevation (Young Mania Rating Scale; YMRS=15) there was reduced negative functional connectivity between the amygdala and ventrolateral PFC during "perceiving" and "labeling" emotion when viewing faces with angry or fearful expressions. Anand et al., (2009), however, reported reduced connectivity during resting state between the amygdala and pregenual anterior cingulate in a small sample of unmedicated patients with bipolar and unipolar illness. This data, in conjunction with the literature, suggests that the nature of the cognitive task may be an important variable in determining which particular prefrontal region is involved in alterations observed in functional connectivity. Taken together, prefrontal-amygdalar circuits appear abnormal with an over abundance of positive connectivity in bipolar illness, even during simple attention tasks.

As others have observed (Strakowski et al., 2005; Drevets et al., 2008), we saw small but significant elevations in mean metabolic activity of both amygdala in bipolars compared to unipolars and controls. It is not clear whether this increased amygdalar activity may be driving the increased positive functional connectivity observed in this study or the result of it. Moreover, further studies are needed to examine whether the functional connectivity of the amygdala returns to normal with various treatment modalities, e.g., thyroxine (T4; Bauer et al., 2005) and 1 Hz rTMS (Speer et al., 2000) which normalize amygdalar over-activity.

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This study adds to existing evidence of amygdala alterations in bipolar illness including: 1) the altered volume of the amygdala, being consistently decreased in children (Chang et al., 2005; Caetano et al., 2004; Blumberg et al., 2003; DelBello et al., 2004) with a trend for increases compared to controls in adults (Strakowski et al., 1999; Altshuler et al., 1998, 2000; Brambilla et al., 2003; Frangou et al., 2002; Pearlson et al., 1997; Velakoulis et al., 2006); 2) abnormal number of amygdalar neurons and related glia (Hamidi et al., 2004; Bowley et al., 2002); and 3) increased amygdalar reactivity to emotional challenges (Benson, 2005; Blumberg, et al., 2005).

The amygdala has been closely linked to the modulation of conditioned fear and other conditioned emotional learning paradigms (LeDoux, 1996; Davis, 1995; Davis et al., 2003). Morgan et al. (1993) found that when the medial prefrontal cortex is lesioned, stimulus-reward associations in the amygdala are difficult to change when reward and punishment contingencies have been reversed. The work of LeDoux (1996) and Rolls et al., 1992) also suggests that the amygdala-medial prefrontal cortex circuit engagement is important for extinction learning and shifting of behavioral strategies. Given the evidence of relative cortical hypofunction in bipolar depression (Ketter et al., 2001) and the loss of normal inverse relationships between the amygdala and prefrontal cortex seen here, one could envision three components lessening the degree of cortical control over amygdala activity in bipolar disorder. Such corticolimbic dysregulation could include: 1) cortical hypofunction itself; 2) amygdala hyperactivity at rest and response to affective stimuli; and 3) inappropriate enhanced positive connectivity or loss of normal inverse reciprocal relationships. To the extent that reciprocal relationships are balancing, modulatory, or exert braking functions of one region upon another, perhaps the loss of normal inverse amygdalar-prefrontal associations in bipolar illness could contribute to the loss of normal affective and behavioral modulation seen during episodes of both mania and depression.

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In contrast, the unipolars exhibited two sets of alterations producing a net effect of reduced positive functional connectivity. First, the metabolism in both amygdalae either lacked or had few positive correlations (R>L) with the rest of the brain and especially the contralateral amygdala, suggesting each amygdala was acting relatively independently. Second, amygdalar metabolic associations with left DLPFC and anterior cingulate were negative even more inverse than in controls.

These findings parallel the reduced amygdalar correlations with the anterior cingulate in depressed unipolars during rest (Lui et al., 2011) and with during emotion-induction (Anand et al., 2005). In addition, they complement decreased connectivity with the DLPFC (Irwin et al., 2004; Dannlowski et al., 2009) also seen with emotion-induction tasks. These amygdalo-prefrontal systems are hypothesized to be critical to the generation of affective states and emotion regulation (Phillips et al., 2003). The current findings suggest that in relatively treatment-resistant patients with major depression these circuits appear to be functioning abnormally even during non-emotive tasks. Amygdalar hypoconnectivity in UPs could yield decreased amygdala influence on other regions, which may be related to the lack of normal affective range seen in this disorder.

Local increases in amygdalar activity in patients with unipolar illness seen by others (Drevets et al., 1992 [CBF]) were not replicated in this study, possibly due to the different populations studied (treatment refractory versus Familial Pure Depression Disorder - FPDD). Alternatively, there may be a lack of coupling between CMRglu and CBF as reported in treatment refractory unipolars, but not bipolars or controls (Dunn et al., 2005).

The amygdala's most robust projections in monkeys are to anterior cingulate (BA 24, 25, 32) and orbitofrontal cortex (BA 14, 13a, and 12) (Amaral and Price, 1984; Price 2003). This pathway, normally inhibitory, shows opposite patterns of positive connectivity in

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bipolar patients compared to controls and heightened inverse connectivity in unipolars. This differential functional connectivity could relate to the putative neurochemistry of these pathways (Öngur and Price, 2000; Drevets 2000, 2003; Strakowski et al., 2005) that may be glutamatergic (Amaral et al., 1992; McDonald, 1996) or cholinergic (Russchen et al., 1985a, b; Amaral et al., 1992). Alternatively, emerging data from diffusion tensor imaging studies suggest that there may be anatomical alterations in connectivity measured by increased fractional anisotropy (FA) or number of reconstructed fibers in the left uncinate fasiculus in patients with bipolar illness compared to controls (Versace et al., 2008; Houenou et al., 2007). How the hyperconnectivity seen in our study relates to the over-reactivity of the amygdala seen during facial emotion recognition tasks remains to be determined. During these tasks, amygdala activity increases in children (Chang, 2005; Leibenluft and Rich, 2008) and adults (Yurgelin-Todd et al., 2000), and may also be related to mood state as amygdala activity increases occur in adult mania (Chen et al., 2005; Altshuler et al., 2005) and decreases in depression (Lennox, et al., 2004; Altshuler et al., 2008b). Examining functional connectivity of the amygdala during these types of emotion tasks as opposed to the emotion-neutral CPT task we utilized would be particularly important.

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Differential Hippocampal Relationships in Bipolar and Unipolar Affective Illness

The metabolic interrelationships of the hippocampus with other brain regions observed in this study were not predicted (Figure 2). Bipolars had fewer significant correlations to other brain areas than those seen for any other ROIs that we examined including the amygdala seen here and as previously reported thalamus, and prefrontal insular, and parietal cortices (Benson et al 2008). Conversely, these unipolars had a greater number of significant correlations correlations of the hippocampus to other brain areas.

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In the bipolar patients, significant differences from controls primarily occurred with posterior areas usually associated with cognitive function, including reduced positive connectivity of the hippocampus with inferior parietal cortex, parahippocampal, posterior cingulate, and cuneus, and reduced negative connectivity with mid-temporal occipital cortex and cerebellum. Decreased activation of the hippocampus during encoding tasks in bipolars compared to controls has been reported (Deckersbach et al., 2005). Whether this connectivity or activation pattern is related to some of the cognitive deficits persisting in euthymic bipolar patients (Thompson et al., 2005; Malhi et al., 2007; Cahill et al 2009; Torres et al., 2010) that are more severe in patients with a greater number of previous episodes (Kapczinski, et al., 2008; Post et al., 2012) deserves further examination.

The unipolars tended to have enhanced (greater than controls) inverse relationships of the hippocampus with prefrontal and temporal regions. These altered hippocampal associative relationships provide further evidence of hippocampal abnormalities revealed by the findings of hippocampal volume deficits in unipolar depression as a function of the number or duration of depressive episodes (Sheline, 2003; Campbell et al., 2004) or the degree of treatment refractoriness (Sheline 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004). This could be a functional correlate of cortical grey or white matter thinning as reported by Peterson and Weissman (2011). The relative hippocampal hyperconnectivity in UPs could reflect a compensatory effect secondary to the volume loss, or conversely could reflect processes driving the volume loss, such as excitotoxicity or apoptosis. The relatively preserved pattern of connectivity of the hippocampus in the UPs vs BPs could also relate to the fewer cognitive deficits compared to BPs.

The preliminary data that the functional connectivity of the amygdala and hippocampus in unipolars differs as a function of laterality is presented here for its potential heuristic value. The amygdalae relationships were skewed in the direction of left-

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more positive and right-more negative connectivity, while the hippocampus showed the opposite pattern of left-negative and right-positive associative predominance. These findings parallel volumetric studies showing that the right amygdala and left hippocampus to be smaller than their contralateral homologous structures (Mervaala et al., 2000). Laterality differences in amygdalar activity and function has been discussed by many investigators (Davidson 2002; Davidson and Irwin, 1999; Morris et al., 1998; Ketter et al 1996) and the functional significance of the connectivity differences remain to be further defined.

Limitations

A number of caveats to the interpretation of these data are warranted. As discussed previously (Benson et al., 2008), these include: the use of a continuous performance task as opposed to an affective challenge test; the unequal sample size of the two patient groups compared to the larger group of controls; and uniformly high degree of treatment resistance in the BPs (Frye et al., 2000), but a less uniform degree of treatment resistance in the UPs. Examining the functional connectivity using other challenge paradigms and in other patient populations is indicated. In addition, whether the connectivity changes observed here are relatively persistent across ill and well states or differ as a function of early versus late in the course of illness requires further examination. Another potential limitation is that the correlative relationships could be confounded by subtle anatomical differences and/or grey/white boundary uncertainties.

However, the distinctly different functional connectivity patterns of the amygdala and hippocampus and their double dissociation in patients with bipolar compared to unipolar disorder suggest that they are not artifacts of anatomical differences or methodological confounds. Nevertheless, how these patterns of metabolic associativity

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delineated by correlation relate to other more anatomically and temporally discrete measures of connectivity with fMRI and DTI deserves further investigation. Finally, how positive versus negative functional correlative relationships observed here with PET relate to excitatory or inhibitory transmitter function and dysfunction is not presently known and requires clarification.

Conclusions and Implications

The striking but preliminary findings of this study suggest that there are quite substantial differences in the functional connectivity of the amygdala and hippocampus between patients unipolar and bipolar disorder. These altered patterns of connectivity were seen whether or not there were baseline local metabolic abnormalities in these areas during the continuous performance task. In bipolars, the enhanced positive associativity of the amygdala with other brain suggests that at any single time-point brain activity across the majority of the brain may be more uniformly activated or deactivated in concert with activity in the amygdala than is normally the case in controls or in patients with unipolar disorder. This positive hyper-connectivity of the amygdala with the rest of the brain could be associated with the unmodulated extremes of affective, behavioral, and cognitive processes commonly observed in bipolar illness. These connectivity abnormalities observed here add to an increasing strong literature indicating that amygdala dysfunction occurs in patients with bipolar disorder.

In contrast, the hippocampus in bipolars was relatively less metabolically coupled with much of the rest of the brain, especially the posterior association areas. This paucity of hippocampal connectivity could be related to the prominent cognitive deficits seen in even euthymic patients with bipolar disorder. This putative relationship of hippocampal

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disconnectivity to cognitive dysfunction remains to be more specifically examined in other patient populations and in those with lesser degrees of treatment-resistance.

Unipolar patients demonstrated essentially opposite amygdala and hippocampal associative patterns compared to the BPs. In UPs the amygdala was less connected than in controls, while the hippocampus showed enhanced connectivity to many other brain regions, and these occurred with laterality differences not seen in the BPs or controls. The origin and functional correlates of these abnormalities require further exploration. It is tempting to speculate that the lack of amygdala connectivity in the UPs could relate to the narrowing of their range of emotions and to anhedonia, while the hyperconnectivity of the hippocampus could account for their relatively preserved cognitive functions and yet their excessive and repetitive depressive ruminations and/or attempts at cortical control of them. Enhanced inverse relationships with prefrontal cortex may exacerbate the normal inhibition needed for appropriate psychosocial functioning, possibly creating a negative bias in their percepts. This particular combination of inverse relationships of the prefrontal cortex that are decreased with the amygdala and increased with hippocampus may help explain their dysfunction in processing and recognizing emotions whether introspectively or in the affect of others. Examining these and other potential correlates of the differential patterns of the amygdala and hippocampal metabolic connectivity to other brain regions will require further study of patients in ill and well states and with the use of a range of cognitive tasks and emotional evocative tasks.

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Figure captions

Figure 1

Amygdalar-prefrontal metabolic relationships were altered in both bipolar (middle panel) unipolar (bottom panel) illness relative to healthy controls. Bilateral (right not illustrated) amygdalae in bipolars correlated significantly more positively with medial prefrontal cortex compared to controls (top panel). In contrast, amygdalar metabolism in unipolars either lacked or was significantly more negative compared to controls with the anterior cingulate and DLPFC, respectively. These amygdalo-prefrontal systems hypothesized to be critical to the generation of affective states and emotion regulation have abnormal functional relationships even in non-emotive tasks in these treatment-resistant patients. Correlations are represented in shades of red and blue to designate positive and negative, correlations, respectively. Numbers indicate the Z plane corresponding to the atlas Talairach and Tourneaux (1988). L=left.

Figure 2

Metabolic correlations of the left hippocampus in both bipolar (middle panel) appeared almost non-existent in stark contrast to the almost exclusively enhanced positive relationships seen in the amygdala, cortex, thalamus, and cerebellum (Benson et al., 2008), and the negative cortical and positive subcortical seen in controls (top panel). Unipolars (bottom panel), on the other hand, exhibit considerable positive and negative relationships with anterior and posterior regions, respectively. This also is in marked contrast to other regions examined thus far. Correlations are represented in shades of red and blue to designate positive and negative, correlations, respectively. Numbers indicate the Z plane corresponding to the atlas Talairach and Tourneaux (1988). L=left.

Figure 3

A schematic summarizing amygdalar and hippocampal relationships observed in patients with bipolar illness (middle) and unipolar illness (right), and healthy controls (left). In bipolars, the functional connectivity of the amygdala is significantly enhanced compared to healthy controls, where most of these target regions displayed here have significantly greater positive correlations. In contrast, the hippocampus of bipolars appears to be significantly reduced functional connectivity. In unipolars, the amygdala has significantly reduced functional connectivity and the hippocampus has significantly stronger negative connectivity, suggesting a double dissociation of amygdala and hippocampal abnormalities in unipolar and bipolar disorder.

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Conflict of Interest. All authors declare they have no conflict of interest in this work.

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Contributors

Brenda E. Benson wrote the manuscript and analyzed the data. Mark W. Willis developed the methods used in the analyses, and analyzed the data for the controls subjects. Terence A. Ketter collected the primary data and recommended analysis methods. Andrew Speer, Tim A. Kimbrell, and Mark S. George collected the primary data. Peter Herscovitch advised on PET imaging acquisition. Robert M. Post, as lab chief, advised on project. All authors contributed to and have approved the final manuscript.

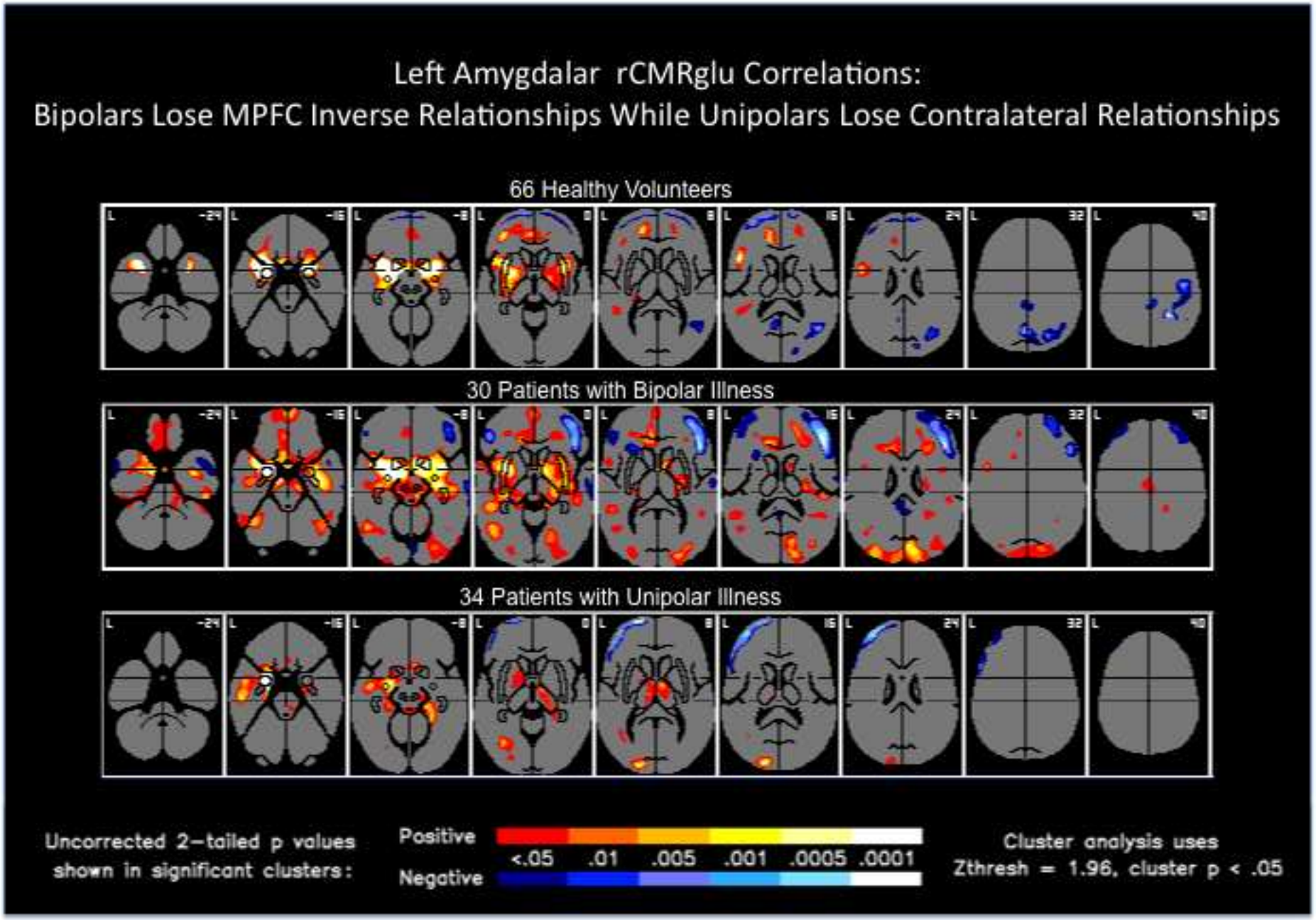
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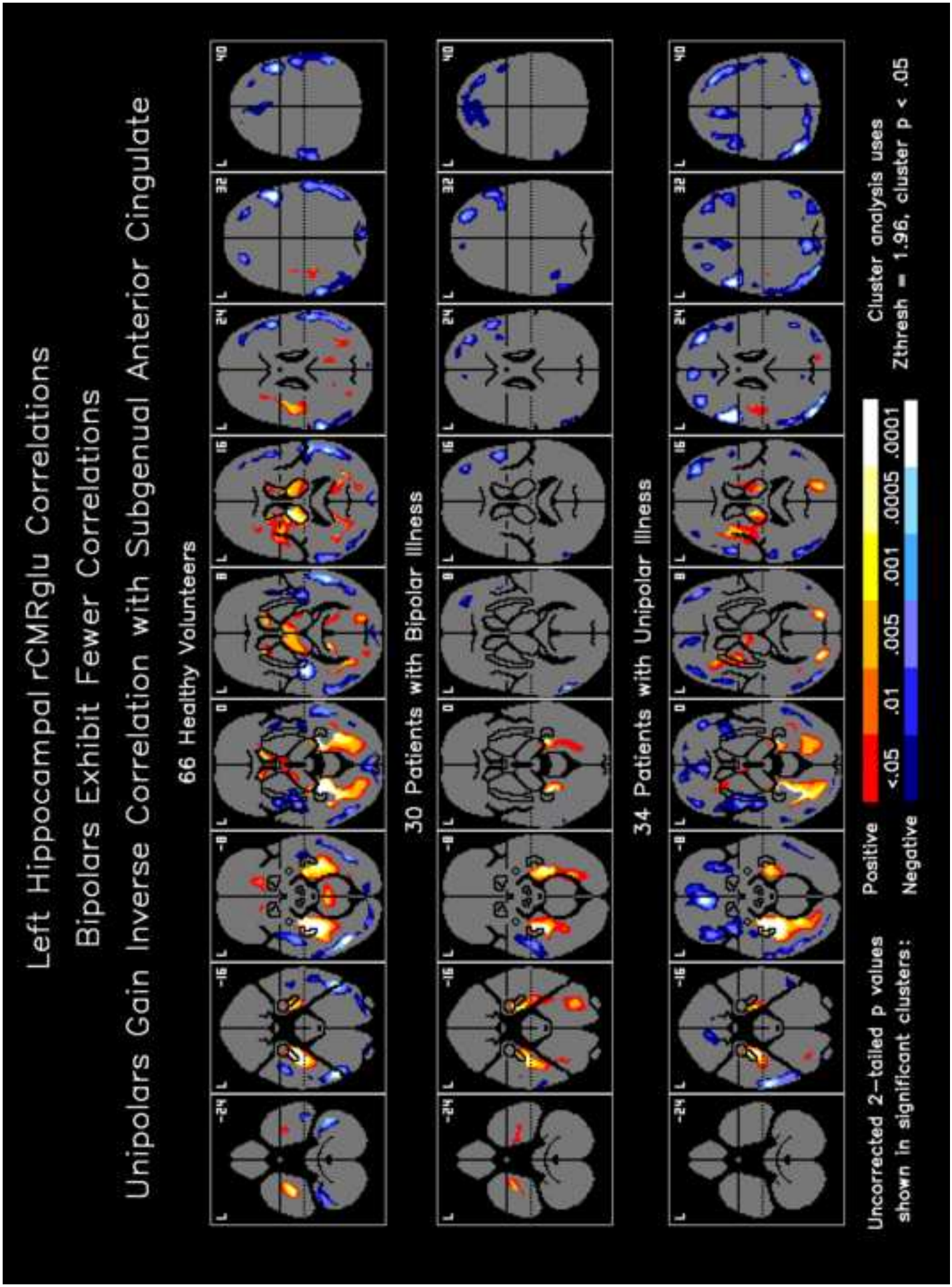
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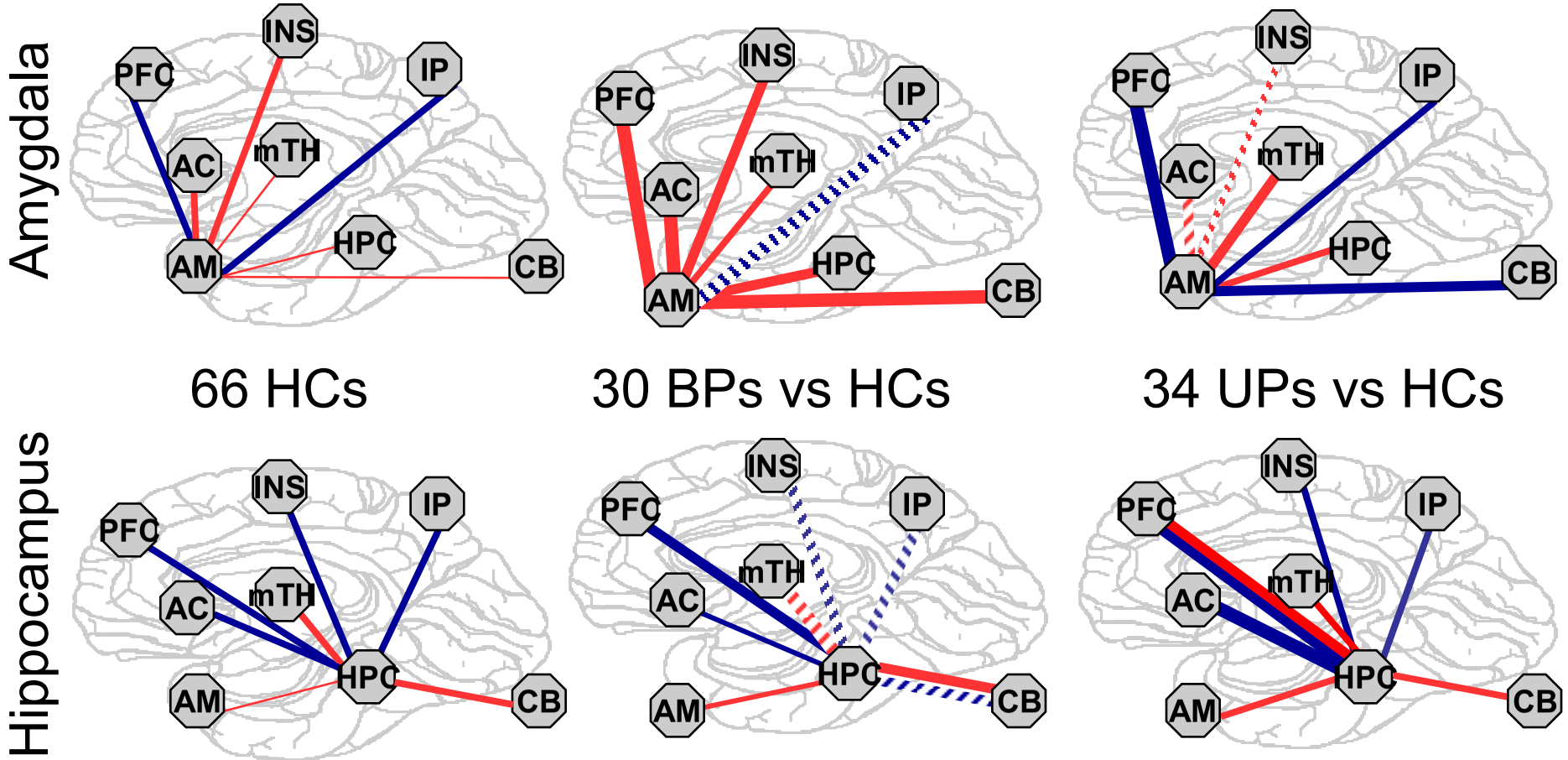
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Figure(s)

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Differential Functional Connectivity in Bipolar and Unipolar Illness



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