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Amygdala Activation and Connectivity to Emotional Processing Distinguishes Asymptomatic Patients With Bipolar Disorders and Unipolar Depression

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ABSTRACT

BACKGROUND: Mechanistically based neural markers, such as amygdala reactivity, offer one approach to addressing the challenges of differentiating bipolar and unipolar depressive disorders independently from mood state and acute symptoms. Although emotion-elicited amygdala reactivity has been found to distinguish patients with bipolar depression from patients with unipolar depression, it remains unknown whether this distinction is traitlike and present in the absence of an acutely depressed mood. We addressed this gap by investigating patients with bipolar disorder (BP) and unipolar major depressive disorder (MDD) in remission.

METHODS: Supraliminal and subliminal processing of faces exhibiting threat, sad, happy, and neutral emotions during functional magnetic resonance imaging was completed by 73 participants (23 BP patients and 25 MDD patients matched for age and gender, number of depressive episodes and severity; 25 age- and gender-matched healthy control subjects). We compared groups for activation and connectivity for the amygdala.

RESULTS: BP patients had lower left amygdala activation than MDD patients during supraliminal and subliminal threat, sad, and neutral emotion processing and for subliminal happy faces. BP patients also exhibited lower amygdala connectivity to the insula and hippocampus for threat and to medial orbitofrontal cortex for happy supraliminal and subliminal processing. BP patients also demonstrated greater amygdala-insula connectivity for sad supraliminal and subliminal face processing. Both patient groups were distinct from control subjects across several measures for activation and connectivity.

CONCLUSIONS: Independent of valence or level of emotional awareness, amygdala activation and connectivity during facial emotion processing can distinguish BP patients and MDD patients. These findings provide evidence that this neural substrate could be a potential trait marker to differentiate these two disorders largely independent of illness state.

Keywords: Amygdala, Bipolar disorders, Depression, Emotion processing, fMRI connectivity, Remission

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In approximately 60% of patients, bipolar disorder (BP) is initially misdiagnosed as major depressive disorder (MDD) (1,2). This is because BP often first manifests in the depressive phase of the illness, and bipolar depression is similar to unipolar major depression in terms of clinical symptoms. This can result in delay in diagnosis of up to a decade, and during this time patients may be subjected to suboptimal treatment with predictably poorer outcomes—compounding the societal and economic burden of this disorder (3,4). It is increasingly evident that existing diagnostic clinical tools are incapable of differentiating the two disorders in their early stages before the development of mania (5–7). Clearly, identifying pathophysiological markers that could reliably differentiate these two disorders would therefore have substantial clinical value.

Both BP and MDD exhibit abnormalities of emotion processing with consistent abnormal activation in the amygdala (8–10). Amygdala responsivity during emotion processing could be a core feature in distinguishing bipolar depression from unipolar depression. Prior studies suggest that both abnormal activation and functional connectivity of the amygdala elicited by negative and positive facial emotions may be a statelike marker for BP (11–14). The focus of these studies has been on the comparison of depressed individuals with BP relative to individuals with MDD (11–13) and individuals with BP in depressed versus remitted states (13,14). However, the

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directionality of effects across these studies has largely been inconsistent. The differences are evident but may be dependent on mood state, in particular depression. It remains unknown whether such discrimination at a neural level persists in individuals in remitted states and could potentially serve as a trait marker for differentiating these disorders in individuals regardless of whether they are depressed. Such a marker could help in better understanding the pathophysiology underlying both these disorders, identify risk factors for developing these disorders, and potentially enable clarity in diagnosis early from onset. Notably, the numbers of studies directly comparing BP and MDD are few, and no previous study has compared patients in euthymic or remitted states.

Amygdala responsivity elicited under conditions of both bottom-up automatic reactivity and top-down evaluation is a promising probe for addressing this gap in knowledge (15). Previous work has shown that MDD is characterized by abnormal amygdala responses both during the automatic reactivity to emotion stimuli (when presented subliminally) and during the more controlled evaluation of these stimuli (when presented supraliminally) (16,17). Amygdala responsivity probed by subliminal emotion stimuli has also been shown to distinguish individuals with BP from individuals with MDD in a depressed state, with individuals with BP exhibiting greater amygdala activation for happy stimuli and lower amygdala activation for sad stimuli (18).

In this study, we examined amygdala responsivity and connectivity in a cohort of individuals with remitted bipolar depression and unipolar depression. To determine whether stimulus valence or level of awareness in processing modulates disease-related activity within the emotion processing network, we employed two functional magnetic resonance imaging (fMRI) tasks that tapped into both supraliminal and subliminal processing of threat, sad, happy, and neutral emotions. Based on previous studies comparing patients with bipolar depression and patients with unipolar depression, we hypothesized amygdala activity and connectivity in response to emotion processing elicited by a range of facial emotions would differentiate the two disorders in remission.

METHODS AND MATERIALS

Participants

This study included 81 participants 18 to 60 years of age: 31 remitted individuals with BP, 25 remitted individuals with MDD, and 25 healthy control (HC) subjects. Participants underwent fMRI scans and testing at the Brain Dynamics Centre, Westmead Institute for Medical Research.

Study Protocol

BP and MDD patients were recruited through general practitioner referrals and clinics in the Western Sydney Local Health District and in the greater Sydney area or through community advertisements. Patients met DSM-IV criteria for bipolar I disorder or MDD and were required to be remitted at time of testing, screened through a series of clinical assessments, including the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (19), Mini-International Neuropsychiatric Interview (20), 17-item Hamilton Depression Rating Scale (HDRS-17) (21), and Depression Anxiety and Stress Scale (DASS) (22). Remission was defined as at least 14 days of being asymptomatic, i.e., no manic episode and a depression severity score of HDRS-17 < 7 for at least 2 weeks before and at the time of testing. BP patients were taking lithium ($n = 15$), antipsychotics ($n = 18$), antidepressants ($n = 6$), anticonvulsants ($n = 9$) or benzodiazepine ($n = 2$), whereas all MDD patients were taking antidepressants (selective serotonin reuptake inhibitors [$n = 13$] and serotonin and norepinephrine reuptake inhibitors [$n = 12$]) at time of testing. HC subjects were recruited from the general community and screened extensively using the Mini-International Neuropsychiatric Interview to ensure they did not meet DSM-IV criteria for any recurrent or nonrecurrent Axis I or other psychiatric disorder. All participants were required to have no previous history of psychiatric diagnoses (other than BP or MDD for the respective patient groups), current or previous substance dependence, history of brain injury, previous loss of consciousness for greater than 10 minutes, self-reported serious medical conditions, or any contraindications for MRI. Eight participants with BP had to be excluded (1 participant was manic during testing, 4 participants did not finish both emotional processing tasks addressed in the study, and 3 participants exhibited excessive motion during the tasks), resulting in 23 BP patients, 25 MDD patients, and 25 HC subjects for analyses. All participants provided informed consent, and the study was conducted in accordance with the ethical guidelines of the institutional review board.

fMRI Acquisition and Task Details

MRI data were acquired on a 3T GE Signa HDx scanner (GE Healthcare, Wauwatosa, WI) at the Department of Radiology, Westmead Hospital, using an eight-channel head coil. fMRI was acquired using echo-planar imaging (repetition time/echo time = 2500/27.5 ms; matrix = 64×64; field of view = 24 cm; flip angle = 90°). Forty slices, each 3.5 mm thick, covered the whole brain in each volume. For each task, 120 volumes were collected with a total scan time of 5 minutes 8 seconds. Specifically, the scan consisted of five tasks (only two, supraliminal and subliminal emotional processing, are reported and analyzed in this article) and a three-dimensional T1-weighted structural MRI scan (repetition time/echo time = 8.3/3.2 ms; flip angle = 11°; inversion time = 500 ms; number of excitations = 1; array spatial sensitivity-encoding technique = 1.5; frequency direction: superior-to-inferior; matrix = 256×256; 180 contiguous 1-mm sagittal slices covering the whole brain; 1-mm^3 voxels).

The details of the emotional processing tasks have been previously described (17,23). Briefly, in the supraliminal emotional processing task, participants passively viewed a series of emotional faces (angry, disgusted, fearful, sad, happy, and neutral) for 500 ms per stimulus, with an interstimulus interval of 750 ms. In the subliminal task, participants were presented with identical faces; however, the stimulus duration was 10 ms and was immediately followed with a neutral face for 150 ms. To control for conscious detection on the basis of perceptual features, the neutral masked faces were offset by 1° in random directions. Prior testing using behavioral psychophysiological testing indicates that presentation of facial stimuli at $\leq$ 20 ms meets signal detection

criteria for discrimination of emotional expression (24). The interstimulus interval within the subliminal task was 1100 ms to ensure that all stimulus presentation was equivalent across conditions and task. Across both tasks, there were a total of 240 stimuli, grouped into blocks of eight faces of the same emotion and repeated five times. In both tasks, participants were instructed to pay attention to each emotion face in order to respond to posttest questions.

fMRI Data Analyses

Data were preprocessed and analyzed using SPM8 (http://www.fil.oin.ucl.ac.uk/spm), as described previously (23) and in detail in the Supplement. Briefly, data were first realigned and unwarped and then screened for motion artifacts using the Artifact Detection Tools (www.nitrc.org/projects/artifact_detect/); normalization parameters to transfer to standard space were estimated; and global signal from cerebrospinal fluid and white matter was removed, followed by smoothing and high-pass filtering. First-level general linear models for each task consisted of regressors representing blood oxygen level–dependent responses for each emotion block and the motion parameters and were used to derive the following contrast images: for threat-related emotions (anger, fear, disgust) versus baseline average (to assess activation and connectivity elicited by signals relevant to direct threat, indirect threat, and threat of contaminants); sad versus baseline (to assess activation and connectivity elicited by signals of potential loss); happy versus baseline (to assess activation and connectivity elicited by signals of potential positive affect and reward); and neutral versus baseline (to assess activation and connectivity elicited by a neutral condition). We chose to evaluate each emotion relative to baseline average (i.e., implicit baseline) as compared with neutral because of recent mounting evidence that neutral faces may not be suitable baseline comparisons (25). However, for comparisons with previous fMRI studies of emotion processing using neutral as baseline, we also evaluated contrasts of each emotion relative to neutral in supplementary analyses. All contrast images were individually normalized to standard space using the warps estimated from the preprocessing steps noted previously, and these normalized contrast maps were then used for all second-level analyses.

Activation Analysis

The primary second-level analysis for both supraliminal and subliminal emotional processing tasks consisted of voxelwise analyses of variance between group (two: BP and MDD) by emotion (six: anger, fear, disgust, sad, happy, neutral) to compare neural activations between the BP and MDD groups. These were conducted in the left and right amygdala regions of interest (ROIs) defined using the Automated Anatomical Labeling atlas (we also performed exploratory whole-brain analyses, reported in Supplement). Post hoc voxelwise analyses for individual emotion contrasts were performed for significant main effects. To control for type I errors, a cluster-level correction procedure was employed. A corrected α level of .05 was estimated employing Monte Carlo simulations using REST AlphaSim software (www.restfmri.net) for an uncorrected height threshold of .05. For the bilateral amygdala ROI, the minimum significant cluster size to maintain the threshold was 27 voxels. Identical voxelwise analyses were also run between patient groups and HC subjects (reported in Supplement).

Connectivity Analysis

Functional connectivity analyses (generalized psychophysiological interactions) (26) were performed using the left amygdala as the seed region, selected based on the group-by-emotion activation findings. The details of the generalized psychophysiological interactions model and analysis are described in the Supplement. As done for the activation analyses, connectivity maps were compared voxelwise in a second-level analysis between the groups using cluster corrections. We evaluated the threat, sad, and neutral emotions in a single analysis of variance and happy emotion contrasts in separate second-level ROI analyses as we tested connectivity differences between groups in specific networks depending on emotion (27). The negative affect network (comprising the subgenual anterior cingulate cortex [sgACC] and pregenual anterior cingulate cortex, bilateral hippocampus, insula, and amygdala) was used for connectivity analyses of the threat, sad, and neutral emotion contrasts. The positive affect network (comprising the dorsal anterior cingulate cortex, medial orbitofrontal cortex [mOFC], bilateral caudate, putamen, and pallidum) was used for the connectivity analysis of the happy emotion contrast. These networks were chosen for their relevance to the neural circuitry of negative and positive emotional processing (27) and ROIs defined using the Automated Anatomical Labeling atlas and meta-analyses (28). The minimum significant cluster size to maintain a corrected α .05 threshold was 71 voxels for the negative affect network and 80 voxels for the positive affect network.

Correlation With Symptoms

β values (averaged) were extracted from the significant activation and connectivity clusters found between BP and MDD groups and were tested for correlations with symptom severity (HDRS-17, DASS, Young Mania Rating Scale scores) using SPSS, version 22 (IBM Corp., Armonk, NY). We examined correlations within each group separately as well as partial correlations with the pooled patient group controlling for diagnostic category. Finally, we also evaluated accuracy of the significant activation and connectivity clusters in classifying the patient groups using backward stepwise (Wald) binary logistic regression analyses and tested generalization of these models using leave-one-out cross-validation statistics.

RESULTS

Table 1 shows the demographic and clinical characteristics for all participants. BP patients were matched to MDD patients for age, gender, number of depressive episodes, and depression severity (based on HDRS-17 and DASS scores). The BP and MDD groups were also matched to HC subjects for age and gender but had higher DASS depression scores than HC subjects ($p < .05$ for two group comparisons). For fMRI comparisons for each patient group relative to HC subjects, we tested in a post hoc analysis if effects also survived controlling for the DASS measures (see Supplemental Results).

Table 1. Participant Demographics and Clinical Characteristics

	BP (n = 23)	MDD (n = 25)	HC (n = 25)	F/χ^2	p Value
Age, Years, Mean ± SD (Range)	33.48 ±13.43 (18–62)	32.83 ± 12.97 (18–62)	34.29 ± 13.31 (18–60)	0.08	NS
Gender, % Female	65.22	64.00	65.22	0.15	NS
DASS Depression, Mean ± SD	8.82 ± 7.76	7.76 ± 5.61	3.89 ± 4.38	3.45	.038 (BP > HC; MDD > HC)
DASS Anxiety, Mean ± SD	6.55 ± 7.26	5.00 ± 5.20	2.22 ± 3.08	3.03	NS
DASS Stress, Mean ± SD	8.00 ± 8.63	4.00 ± 4.09	6.39 ± 4.71	2.52	NS
No. Depressive Episodes, Mean ± SD (Range)	16.95 ± 31.35 (1–99)	12.08 ± 15.93 (1–60)	N/A	0.45	NS
No. Manic Episodes, Mean ± SD	14.68 ± 28.37 (1–99)	–	–	–	–
HDRS-17, Mean ± SD	5.22 ± 6.11	5.6 ± 3.45	N/A	0.07	NS
YMRS, Mean ± SD	2.09 ± 2.58	–	–	–	–
Lithium, n (%)	15 (65)	–	–	–	–
Anticonvulsant, n (%)	9 (39)	–	–	–	–
Antidepressant, n (%)	6 (26)	25 (100)	–	–	–
Antipsychotic, n (%)	16 (69)	–	–	–	–
Benzodiazepine, n (%)	1 (4)	–	–	–	–
Supraliminal Task—Motion Outliers, Mean ± SD	6.87 ± 7.38	3.48 ± 3.98	3.84 ± 5.78	2.41	NS
Subliminal Task—Motion Outliers, Mean ± SD	6.48 ± 8.36	5.56 ± 5.86	4.04 ± 4.21	0.920	NS

BP, bipolar disorder; DASS, Depression Anxiety and Stress Scale; HC, healthy control; HDRS-17, 17-item Hamilton Depression Rating Scale; MDD, major depressive disorder; N/A, not applicable; NS, not significant; YMRS, Young Mania Rating Scale.

Activation Results

Supraliminal Faces Task. BP patients were found to have significant hypoactivation relative to MDD patients in the left amygdala (significant main effect of group across all emotions). This effect was significant for threat (main effect of group across threat emotions), sad, and neutral faces (Table 2 and Figure 1A). There were no significant differences in the amygdala for happy faces. When we unpacked the main effect for threat, this profile of relative hypoactivation for BP versus MDD was apparent for the individual emotional expressions of disgust and fear (details in Supplemental Results). Neither BP patients nor MDD patients differed significantly from HC subjects for supraliminal face processing.

Subliminal Faces Task. Similar to supraliminal processing, BP patients had a significant hypoactivation in the left amygdala compared with MDD patients (significant main effect of group across emotions). In post hoc comparisons, this effect was consistently significant for each of the individual emotions (Table 2 and Figure 1B): main effect of group for threat faces and for the individual threat emotions (anger, fear, disgust), sad, neutral, and happy faces. In contrast to the supraliminal processing, BP patients had significantly reduced amygdala

Table 2. Differences in Amygdala Activation for Both Supraliminal and Subliminal Emotional Face Processing for Euthymic BP and Remitted MDD Groups

Emotion	Direction	Region of Increased Activation	MNI Space X	MNI Space Y	MNI Space Z	Cluster Size	z Score	p Value
Supraliminal								
All emotions	Main effect (BP < MDD)	L amygdala	−24	−2	−28	31	2.81	.003
Threat	Main effect (BP < MDD)	L amygdala	−24	0	−28	32	2.67	.004
Sad	BP < MDD	L amygdala	−24	0	−28	34	2.73	.003
Happy	BP vs. MDD	NS	NS	NS	NS	NS	NS	NS
Neutral	BP < MDD	L amygdala	−24	0	−28	50	3.02	.001
Subliminal								
All emotions	Main effect (BP < MDD)	L amygdala	−22	−6	−18	193	3.74	< .001
Threat	Main effect (BP < MDD)	L amygdala	−22	−4	−18	197	4.08	< .001
Sad	BP < MDD	L amygdala	−20	−4	−18	196	3.51	< .001
Happy	BP < MDD	L amygdala	−22	−4	−18	163	3.23	.001
Neutral	BP < MDD	L amygdala	−22	−4	−18	160	3.13	.001

Effects significant at cluster-level correction ($p < .05$) are shown.
BP, bipolar disorder; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; NS, not significant.

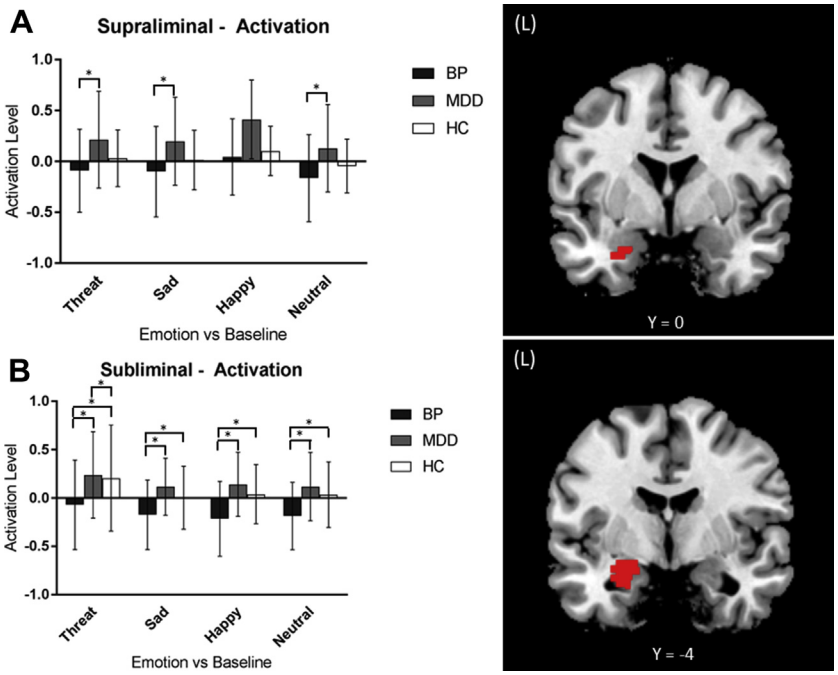

Figure 1. Amygdala activation levels during supraliminal **(A)** and subliminal **(B)** emotion face processing for euthymic bipolar disorder (BP), remitted major depressive disorder (MDD), and healthy control (HC) groups. Brain image shows significant amygdala activation for the main effect of emotion comparing the BP and MDD groups (cluster-level corrected $p < .05$). Bars represent mean activation for significant clusters for different emotions, and error bars represent standard deviations. *Significant group differences from voxelwise post hoc analyses. L, left.

activation across all emotions, whereas MDD patients had greater activation only in the threat emotions relative to HC subjects.

Connectivity Results

We evaluated connectivity related to only the left amygdala based on the activation findings in this region.

Supraliminal Faces Task. For the negative affect network, there was a significant main effect of group for connectivity of the left amygdala with the sgACC, right amygdala, and right hippocampus and a significant group-by-negative emotions interaction for connectivity with the insula (Table 3 and Figure 2A). Similarly, for the threat emotions, a main effect of group and group-by-emotion interaction was also observed for the right hippocampus and insula, respectively. However, between-group differences in connectivity to sgACC for any emotion and to any of the regions for the individual threat emotions did not meet the significance threshold in post hoc comparisons (Supplemental Results). For the other emotions, the BP group had relatively lower amygdala-hippocampus connectivity for sad and neutral faces and lower left-right amygdala connectivity for neutral faces compared with both the MDD group and HC subjects. MDD patients also had greater amygdala-hippocampus connectivity for sad faces than HC subjects. In contrast, amygdala-insula connectivity for sad faces was relatively greater in BP patients than MDD patients, but both groups were not distinct from HC subjects.

For the positive affect network, the BP group had lower left amygdala connectivity than the MDD group with the mOFC, left putamen, and left caudate for happy faces (Table 3 and Figure 2B). Compared with HC subjects, the BP group also had significantly lower amygdala connectivity to the left putamen, whereas MDD had greater amygdala-mOFC connectivity relative to HC subjects.

Subliminal Faces Task. For the negative affect network, there was a significant main effect of group and group-by-negative emotion interactions for connectivity to the pregenual anterior cingulate cortex, bilateral insula, and right hippocampus (Table 3 and Figure 3A). Only group main effects were also observed for the sgACC and right amygdala. Similarly, main group and interaction effects for threat emotions were significant for the right hippocampus and insula. Post hoc analyses revealed that the BP group had amygdala hypoconnectivity to insula and hippocampus mainly to anger and disgust threat emotions relative to MDD (Supplemental Results). This amygdala-insula and amygdala-hippocampus hypoconnectivity for threat also distinguished BP patients from HC subjects, whereas MDD patients were similar to HC subjects. BP patients also had lower left-right amygdala connectivity for sad faces and lower amygdala-hippocampus connectivity for neutral faces compared with MDD patients. Similar to the supraliminal sad faces, amygdala-insula connectivity for subliminal sad faces was relatively greater in BP patients than MDD patients. Connectivity for sad and neutral faces did not distinguish either of the patient groups from HC subjects.

For the positive affect network, the BP group had lower left amygdala connectivity than the MDD group, with the mOFC consistent with that observed for supraliminal

Table 3. Differences in Left Amygdala Connectivity in Negative and Positive Affect Networks for Both Supraliminal and Subliminal Emotional Face Processing for Euthymic BP and Remitted MDD Groups

			MNI Space					
Emotion	Direction	Region	X	Y	Z	Cluster Size	*z* Score	*p* Value
Supraliminal								
Negative Affect Network								
Negative affect	Main effect (BP > MDD)	sgACC	4	26	−14	81	2.33	.01
	Main effect (BP > MDD)	R amygdala	28	0	−18	137	2.78	.003
	Main effect (BP < MDD)	R hippocampus	30	−24	−8	174	2.26	.012
	Interaction	L insula	−36	−2	4	158	2.27	.012
Threat	Main effect (BP < MDD)	R hippocampus	34	−24	−8	95	2.35	.009
	Interaction	L insula	−32	16	−18	75	2.41	.008
Sad	BP > MDD	R insula	44	22	0	369	2.63	.004
	BP < MDD	L hippocampus	−30	−20	−14	150	2.68	.004
	BP < MDD	R hippocampus	24	−16	−12	114	2.28	.011
Neutral	BP < MDD	R amygdala	28	−2	−18	194	3.26	.001
	BP < MDD	R hippocampus	28	−6	−18	223	2.77	.003
Positive Affect Network								
Happy	BP < MDD	mOFC	0	56	−2	256	2.86	.002
	BP < MDD	L caudate	−12	4	20	119	2.64	.004
	BP < MDD	L putamen	−26	−6	−8	110	2.66	.005
Subliminal								
Negative Affect Network								
Negative affect	Main effect (BP < MDD)	pgACC	−2	48	4	241	2.72	.003
	Main effect (BP < MDD)	sgACC	2	24	−8	90	1.95	.026
	Main effect (BP < MDD)	L insula	−38	20	2	302	2.15	.016
	Main effect (BP < MDD)	R insula	40	10	6	77	2.2	.014
	Main effect (BP < MDD)	R amygdala	26	0	−16	95	2.07	.019
	Main effect (BP < MDD)	R hippocampus	38	−30	−8	305	2.97	.002
	Interaction	pgACC	0	48	8	138	2.53	.006
	Interaction	L insula	−36	4	−6	879	3.44	< .001
	Interaction	R insula	36	14	−6	872	3.92	< .001
	Interaction	R hippocampus	32	−20	−8	184	3.44	< .001
Threat	Main effect (BP < MDD)	L insula	−38	18	2	690	2.99	.001
	Main effect (BP < MDD)	R insula	40	10	6	652	3.28	.001
	Main effect (BP < MDD)	R hippocampus	28	−20	−10	435	3.2	.001
	Interaction	L insula	−38	−4	−2	519	2.8	.003
	Interaction	R insula	34	18	−6	244	2.93	.002
Sad	BP > MDD	L insula	−30	10	−20	80	3.23	.001
	BP < MDD	R amygdala	28	−2	−22	92	2.9	.002
Neutral	BP < MDD	R hippocampus	22	−36	4	129	2.44	.007
Positive Affect Network								
Happy	BP < MDD	mOFC	2	48	0	82	2.16	.015

Effects significant at cluster-level correction ($p < .05$) are shown.

BP, bipolar disorder; L, left; MDD, major depressive disorder; MNI, Montreal Neurological Institute; mOFC, medial orbitofrontal cortex; pgACC, pregenual anterior cingulate cortex; R, right; sgACC, subgenual anterior cingulate cortex.

emotional processing (Table 3 and Figure 3B). However, both patient groups were similar in connectivity relative to HC subjects.

Correlation With Symptoms

Correlational analyses between mean β weights extracted from the significant activation and connectivity clusters with HDRS-17, DASS, and Young Mania Rating Scale symptom scores were not significant in either BP or MDD groups or the pooled patient group controlling for diagnostic categorization.

Diagnostic Classification Analyses

To determine whether group differences in activation and connectivity could predict patient diagnoses, we ran three backward stepwise (Wald) binary logistic regression analyses—in two regressions using the significant

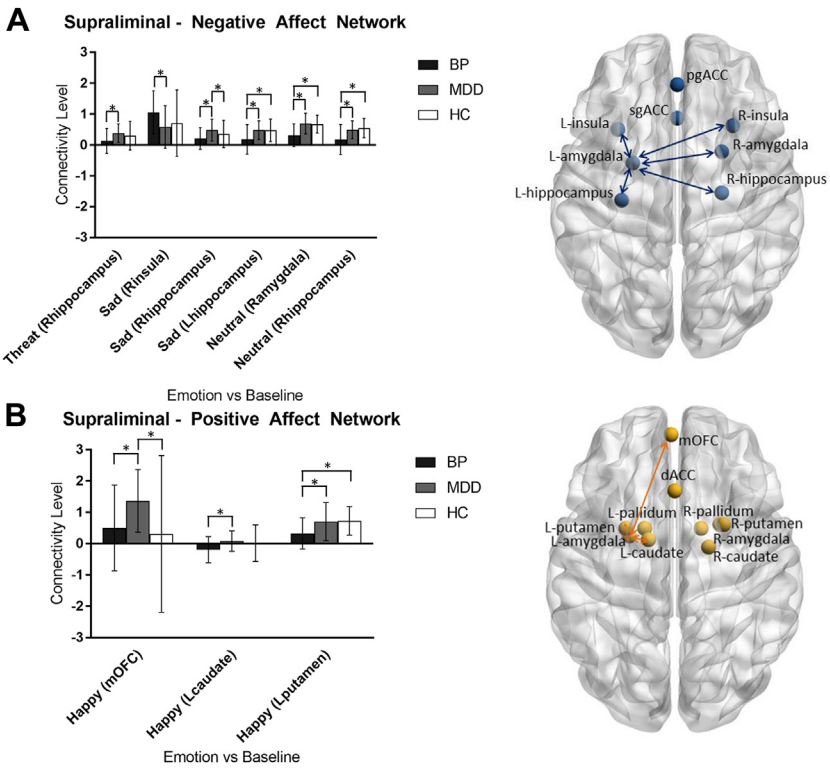

Figure 2. Left amygdala connectivity in the negative affect network **(A)** and positive affect network **(B)** during supraliminal emotion face processing for euthymic bipolar disorder (BP), remitted major depressive disorder (MDD), and healthy control (HC) groups. Only facial emotions with significant connectivity differences are shown in the plot. Bars represent mean activation for significant clusters for different emotions, and error bars represent SD. Brain image shows nodes (spheres) of the negative and positive affect network and connections (arrows) that were significant between the BP and MDD groups. *Significant group differences. dACC, dorsal anterior cingulate cortex; L, left; mOFC, medial orbitofrontal cortex; pgACC, pregenual anterior cingulate cortex; R, right; sgACC, subgenual anterior cingulate cortex.

supraliminal and subliminal results (activation and connectivity) separately, and in the third regression using only the significant supraliminal and subliminal regions from the first two backward stepwise regressions. All three models had a cross-validated accuracy greater than 80% (Table 4). The supraliminal model comprising left amygdala activation for fear and left amygdala connectivity to mOFC and left caudate for happy, to the right amygdala for neutral, and to right insula for sad emotions had the highest classification accuracy of 88.6% (86.4% sensitivity; 90.9% specificity).

DISCUSSION

This study demonstrates that amygdala functional activation and connectivity differ between BP and MDD in remission. Participants with BP had a profile of hypoactivation in the left amygdala relative to participants with MDD that was consistent across level of awareness in emotion processing (i.e., at both supraliminal and subliminal) and type of facial emotions. Relative to participants with MDD, participants with BP also exhibited lower amygdala-insula and amygdala-hippocampus connectivity for the threat emotions and greater amygdala-insula connectivity for the sad emotions for both subliminal and supraliminal processing. Participants with BP also had lower connectivity between the amygdala and the mOFC for viewing of both supraliminal and subliminal happy faces. These findings suggest that differences in amygdala activation and connectivity during processing of facial emotions persist beyond the depressed and manic states and potentially could be a trait marker for differentiating these disorders.

Clinically it is difficult to distinguish between BP and MDD because of the higher prevalence of depressive symptoms relative to hypomanic and manic symptoms during the course of BP and the presence of subthreshold manic symptoms during a depressive episode (29). This problem has led to studies that have evaluated patients with bipolar and unipolar depression particularly during the depressed state (5). Emotion processing is a core problem underlying both of these disorders, and amygdala reactivity and connectivity during facial emotion processing has been identified as a possible state marker to distinguish these disorders (11,13,18,30). However, the directionality of effects across these studies has largely been inconsistent, with studies reporting greater amygdala activation in BPs for happy and fear processing (18,30), lower activation for angry emotions (11), and bidirectional effects (13,18) as well as no differences relative to MDD (11) for sad emotions. We observed lower left amygdala activation for participants with BP relative to participants with MDD in remission. This pattern was consistent across different emotions and during both conscious and nonconscious levels of emotional processing. While amygdala activity during both supraliminal and subliminal facial emotion processing for both BP and MDD cohorts in acute states is widely reported to be different relative to healthy individuals (8,9), there are only a few, mainly inconsistent, reports for asymptomatic cohorts. Euthymic patients with BP are found to have reduced

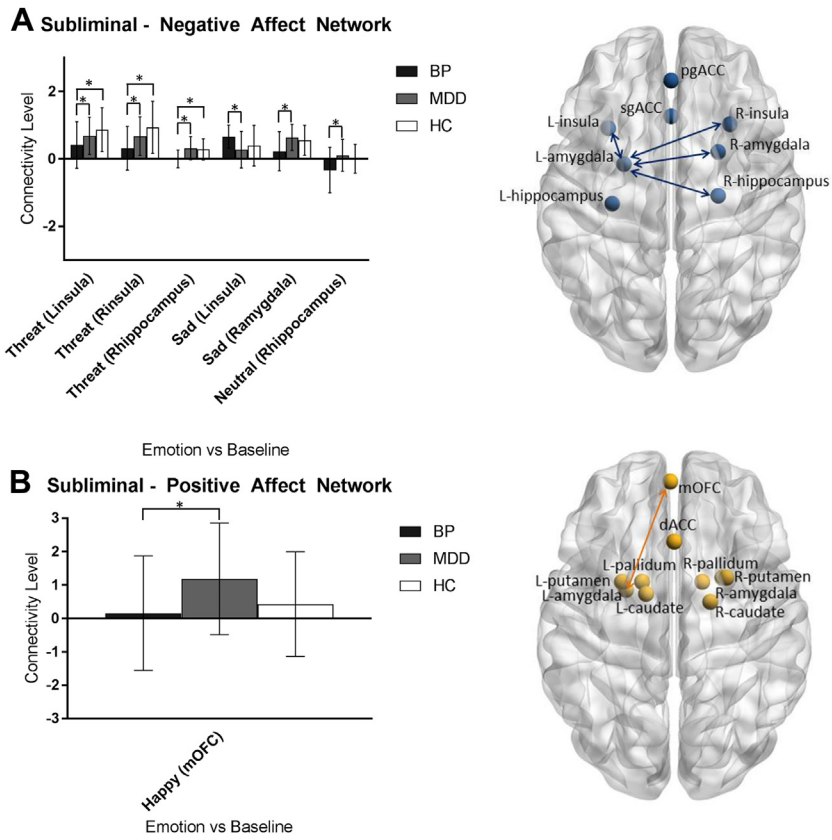

Figure 3. Left amygdala connectivity in the negative affect network **(A)** and positive affect network **(B)** during subliminal emotion face processing for euthymic bipolar disorder (BP), remitted major depressive disorder (MDD), and healthy control (HC) groups. Only facial emotions with significant connectivity differences are shown in the plot. Bars represent mean activation for significant clusters for different emotions, and error bars represent SD. Brain image shows nodes (spheres) of the negative and positive affect network and connections (arrows) that were significant between the BP and MDD groups. *Significant group differences. dACC, dorsal anterior cingulate cortex; L, left; mOFC, medial orbitofrontal cortex; pgACC, pregenual anterior cingulate cortex; R, right; sgACC, subgenual anterior cingulate cortex.

amygdala activity relative to patients in manic states (31), but differences relative to healthy individuals have largely been inconsistent (8). We observed normalized amygdala activation across different emotions only for supraliminal processing, whereas subliminal processing abnormalities, particularly for threat emotions, persist in both patient groups in remission.

The remitted BP group also had lower connectivity relative to MDD to the insula and hippocampus for negative emotions (except for sad stimuli for insula where an opposite pattern was observed) and to the mOFC and striatal brain regions for the happy stimuli (only for supraliminal). The differential direction of connectivity related to the insula, and particularly for sad versus other emotions, may provide some insight into core differences between the two disorders. The insula is regarded as a region strongly underpinning processing of interoceptive states (32), with interconnections with regions associated with the experience of emotion (33) and to hubs of the default mode, cognitive control, and corticostriatal networks (34). Hence, it is crucial in the integration of stimulus-driven bottom-up interoceptive signals with top-down predictions to generate a current emotion awareness state. Clinically, individuals with MDD and individuals with BP exhibit heightened interoceptive awareness, which affects their ability to filter exogenous and endogenous stimuli for adaptive regulation—e.g., increase in negative self-focused thought or rumination in individuals with MDD that impairs shifting from internally focused to externally focused attention or an increase in energy in individuals with BP that prevents regulating from rewarding or threatening cues. This inability for top-down regulation might be reflective of the reduced amygdala-insula connectivity observed for sad stimuli in MDD and that for other emotions in BP (which was also distinct from HC subjects), reflecting their respective core symptoms. Support for this interpretation can be found in the work of Ellard *et al.* (35), who also found differences in resting-state functional connectivity between the insula and the inferior parietal node of the frontoparietal executive brain network to distinguish unipolar and bipolar depressed participants and other reports of differential resting connectivity related to both the amygdala and the insula (36,37). Further, reduced insula and amygdala volumes in individuals at ultra-high risk for the development of psychosis who then subsequently transition to BP, as compared with those who do not, lends further support to the involvement of both of these regions as risk trait markers for BP (38). Consistent with earlier observations in depressed versus remitted BP cohorts (14), we also found reduced connectivity between the amygdala and mOFC during supraliminal happy stimuli for BP relative to MDD, which was characteristic in differentiating participants with remitted MDD but not participants with remitted BP from HC subjects.

There are a number of limitations to this study. First, the study did not involve BP patients or MDD patients who were either currently depressed or, for BP patients, who had current manic or hypomanic symptoms. Studying patient groups across both acute and asymptomatic states is necessary to confirm trait

Table 4. Classification Models to Classify BP and MDD Patient Groups: Regression and Cross-Validation Analyses

		Overall Model Summary		Model Parameters		Prediction Accuracy (Cross-Validated Accuracy)		
Emotion	Measure	χ^2	*p*	β	*p*	Accuracy	Specificity	Sensitivity
Model 1: Supraliminal								
Full model		42.89	.000			95.5% (88.6%)	90.9% (90.9%)	93.2% (86.4%)
Fear	L amygdala			−2.59	.037			
Happy	mOFC[a]			−1.82	.066			
Happy	L caudate[a]			−5.46	.023			
Neutral	R amygdala[a]			−7.17	.015			
Sad	R insula[a]			3.43	.041			
Model 2: Subliminal								
Full model		35.83	.000			81.8% (79.5%)	81.8% (81.8%)	81.8% (77.2%)
Anger	L amygdala			−7.91	.008			
Disgust	R insula[a]			−1.96	.038			
Sad	L insula[a]			−3.64	.02			
Sad	R amygdala[a]			6.09	.02			
Model 3: Supraliminal + Subliminal								
Full model		34.02	.000			86.4% (81.8%)	86.4% (77.2%)	86.4% (86.4%)
Happy	mOFC[a]			−1.19	.049			
Anger	L amygdala			−6.64	.008			
Sad	L insula[a]			−4.10	.004			
Sad	R amygdala[a]			4.68	.022			

BP, bipolar disorder; L, left; MDD, major depressive disorder; mOFC, medial orbitofrontal cortex; R, right.
[a]Connectivity measures.

markers. We did find some of our measures to be correlated with number of episodes and illness age of diagnosis (Supplemental Results) and different relative to HC subjects, which lends some support for traitlike characteristics. Second, we recognize that many of our patients were taking medication at the time of scanning and that the variety of treatments precludes us from parsing out their potential effects, but at the same time it is important to note that all patients had recovered or were asymptomatic and were established on therapy—therefore, the pharmacological effects of medications were likely to be minimal in terms of direct effects and any alterations to emotion regulatory processes per se. We did not observe medication effects on neural measures differentiating the two disorders (Supplemental Results). However, future studies wherever possible should focus on individuals with recent diagnoses who are treatment-naïve to rule out these effects. Third, to evaluate diagnostic clinical value, the neural substrates from our study should be tested for accuracy in classification and replication using an independent cohort. We observed almost 89% cross-validated (internal leave-one-out replication) accuracy (86% sensitivity; 91% specificity) for our measures in classifying the two patient groups, which provides preliminary support that these measures could have promising clinical value. Finally, sample sizes in each patient group are relatively small, and considering that a liberal cluster-corrected statistical threshold was used, our results warrant replication in larger cohorts.

In summary, our study found that amygdala reactivity and connectivity in response to emotion processing elicited by a range of facial emotions successfully differentiated BP and MDD. This effect was mostly independent of the level of awareness in emotion processing and type of facial emotions. Importantly, both patient groups were nonsymptomatic, suggesting that neural substrates for emotion processing may persist largely independent of illness state and could possibly serve as a trait marker that distinguishes these disorders.

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REFERENCES

1. Hirschfeld RM, Lewis L, Vornik LA (2003): Perceptions and impact of bipolar disorder: How far have we really come? Results of the National Depressive and Manic-Depressive Association 2000 survey of individuals with bipolar disorder. J Clin Psychiatry 64:161–174.
2. Dudek D, Siwek M, Zielinska D, Jaeschke R, Rybakowski J (2013): Diagnostic conversions from major depressive disorder into bipolar disorder in an outpatient setting: Results of a retrospective chart review. J Affect Disord 144:112–115.
3. Fritz K, Russell AMT, Allwang C, Kuiper S, Lampe L, Malhi GS (2017): Is a delay in the diagnosis of bipolar disorder inevitable? Bipolar Disord 19:396–400.
4. Kupka RW, Altshuler LL, Nolen WA, Suppes T, Luckenbaugh DA, Leverich GS, *et al.* (2007): Three times more days depressed than manic or hypomanic in both bipolar I and bipolar II disorder. Bipolar Disord 9:531–535.
5. Cardoso de Almeida JR, Phillips ML (2013): Distinguishing between unipolar depression and bipolar depression: Current and future clinical and neuroimaging perspectives. Biol Psychiatry 73:111–118.
6. Phillips ML, Kupfer DJ (2013): Bipolar disorder diagnosis: Challenges and future directions. Lancet 381:1663–1671.
7. Smith DJ, Craddock N (2011): Unipolar and bipolar depression: Different of the same? Br J Psychiatry 199:272–274.
8. Townsend J, Altshuler LL (2012): Emotion processing and regulation in bipolar disorder: A review. Bipolar Disord 14:326–339.
9. Stuhrmann A, Suslow T, Dannlowski U (2011): Facial emotion processing in major depression: A systematic review of neuroimaging findings. Biol Mood Anxiety Disord 1:10.
10. Delvecchio G, Fossati P, Boyer P, Brambilla P, Falkai P, Gruber O, *et al.* (2012): Common and distinct neural correlates of emotional processing in bipolar disorder and major depressive disorder: A voxel-based meta-analysis of functional magnetic resonance imaging studies. Eur Neuropsychopharmacol 22:100–113.
11. Fournier JC, Keener MT, Almeida J, Kronhaus DM, Phillips ML (2013): Amygdala and whole-brain activity to emotional faces distinguishes major depressive disorder and bipolar disorder. Bipolar Disord 15:741–752.
12. Almeida JR, Versace A, Mechelli A, Hassel S, Quevedo K, Kupfer DJ, *et al.* (2009): Abnormal amygdala-prefrontal effective connectivity to happy faces differentiates bipolar from major depression. Biol Psychiatry 66:451–459.
13. Almeida JR, Versace A, Hassel S, Kupfer DJ, Phillips ML (2010): Elevated amygdala activity to sad facial expressions: A state marker of bipolar but not unipolar depression. Biol Psychiatry 67:414–421.
14. Versace A, Thompson WK, Zhou D, Almeida JR, Hassel S, Klein CR, *et al.* (2010): Abnormal left and right amygdala-orbitofrontal cortical functional connectivity to emotional faces: State versus trait vulnerability markers of depression in bipolar disorder. Biol Psychiatry 67:422–431.
15. Williams LM, Liddell BJ, Kemp AH, Bryant RA, Meares RA, Peduto AS, *et al.* (2006): Amygdala-prefrontal dissociation of subliminal and supraliminal fear. Hum Brain Mapp 27:652–661.
16. Suslow T, Konrad C, Kugel H, Rumstadt D, Zwitserlood P, Schoning S, *et al.* (2010): Automatic mood-congruent amygdala responses to masked facial expressions in major depression. Biol Psychiatry 67:155–160.
17. Williams LM, Korgaonkar MS, Song YC, Paton R, Eagles S, Goldstein-Piekarski A, *et al.* (2015): Amygdala reactivity to emotional faces in the prediction of general and medication-specific responses to antidepressant treatment in the randomized iSPOT-D trial. Neuropsychopharmacology 40:2398–2408.
18. Grotegerd D, Stuhrmann A, Kugel H, Schmidt S, Redlich R, Zwanzger P, *et al.* (2014): Amygdala excitability to subliminally presented emotional faces distinguishes unipolar and bipolar depression: An fMRI and pattern classification study. Hum Brain Mapp 35:2995–3007.
19. First MB, Spitzer RL, Gibbon M, Williams JBW (2010): Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P). New York: Biometrics Research, New York State Psychiatric Institute.
20. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, *et al.* (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59(Suppl 20):22–33; quiz 34–57.
21. Hamilton M (1960): A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62.
22. Lovibond SH, Lovibond PF (1995): Manual for the Depression Anxiety Stress Scales, 2nd ed. Sydney: Psychology Foundation.
23. Korgaonkar MS, Grieve SM, Etkin A, Koslow SH, Williams LM (2013): Using standardized fMRI protocols to identify patterns of prefrontal circuit dysregulation that are common and specific to cognitive and emotional tasks in major depressive disorder: First wave results from the iSPOT-D study. Neuropsychopharmacology 38:863–871.
24. Williams LM, Liddell BJ, Rathjen J, Brown KJ, Gray J, Phillips M, *et al.* (2004): Mapping the time course of nonconscious and conscious perception of fear: An integration of central and peripheral measures. Hum Brain Mapp 21:64–74.
25. Filkowski MM, Haas BW (2017): Rethinking the use of neutral faces as a baseline in fMRI neuroimaging studies of Axis-I psychiatric disorders. J Neuroimaging 27:281–291.
26. McLaren DG, Ries ML, Xu G, Johnson SC (2012): A generalized form of context-dependent psychophysiological interactions (gPPI): A comparison to standard approaches. Neuroimage 61:1277–1286.
27. Williams LM (2016): Precision psychiatry: A neural circuit taxonomy for depression and anxiety. Lancet Psychiatry 3:472–480.
28. Liu X, Hairston J, Schrier M, Fan J (2011): Common and distinct networks underlying reward valence and processing stages: A meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev 35:1219–1236.
29. Nusslock R, Frank E (2011): Subthreshold bipolarity: Diagnostic issues and challenges. Bipolar Disord 13:587–603.
30. Lawrence NS, Williams AM, Surguladze S, Giampietro V, Brammer MJ, Andrew C, *et al.* (2004): Subcortical and ventral prefrontal cortical neural responses to facial expressions distinguish patients with bipolar disorder and major depression. Biol Psychiatry 55:578–587.
31. Chen CH, Lennox B, Jacob R, Calder A, Lupson V, Bisbrown-Chippendale R, *et al.* (2006): Explicit and implicit facial affect recognition in manic and depressed states of bipolar disorder: A functional magnetic resonance imaging study. Biol Psychiatry 59:31–39.
32. Craig AD (2009): How do you feel—now? The anterior insula and human awareness. Nat Rev Neurosci 10:59–70.
33. Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, *et al.* (2000): Subcortical and cortical brain activity during the feeling of self-generated emotions. Nat Neurosci 3:1049–1056.
34. Sridharan D, Levitin DJ, Menon V (2008): A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. Proc Natl Acad Sci U S A 105:12569–12574.
35. Ellard KK, Zimmerman JP, Kaur N, Van Dijk KRA, Roffman JL, Nierenberg AA, *et al.* (2018): functional connectivity between anterior insula and key nodes of frontoparietal executive control and salience networks distinguish bipolar depression from unipolar depression and healthy control subjects. Biol Psychiatry Cogn Neurosci Neuroimaging 3:473–484.
36. Ambrosi E, Arciniegas DB, Madan A, Curtis KN, Patriquin MA, Jorge RE, *et al.* (2017): Insula and amygdala resting-state functional connectivity differentiate bipolar from unipolar depression. Acta Psychiatr Scand 136:129–139.
37. Li M, Das T, Deng W, Wang Q, Li Y, Zhao L, *et al.* (2017): Clinical utility of a short resting-state MRI scan in differentiating bipolar from unipolar depression. Acta Psychiatr Scand 136:288–299.
38. Bechdolf A, Wood SJ, Nelson B, Velakoulis D, Yucel M, Takahashi T, *et al.* (2012): Amygdala and insula volumes prior to illness onset in bipolar disorder: A magnetic resonance imaging study. Psychiatry Res 201:34–39.