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Functional connectivity during masked and unmasked face emotion processing in bipolar disorder

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Abstract

Little is known regarding the neural connectivity and correlates during automatic, *unconscious* face emotion processing in individuals with bipolar disorder (BD). In this study, 14 adults with BD and 14 healthy volunteers (HV) underwent fMRI scanning while completing an affective priming task with unconsciously perceived and consciously perceived faces (angry, happy, neutral, blank oval). We found that, regardless of awareness level and emotion types, BD patients exhibited *diminished* functional connectivity between amygdala and ventromedial prefrontal cortex (vmPFC) compared to HV. This connectivity finding is present in the absence of activation differences in amygdala. In addition, in medial frontal gyrus, BD patients displayed *greater* activation while HV displayed *less* activation to angry and neutral faces compared to no face. These results suggest that aberrant amygdala-vmPFC connectivity and neural dysfunction in areas implicated in appraisal and expression of emotions (medial frontal gyrus) may be the pathophysiological correlates of emotional processing in BD regardless of awareness level.

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CONTRIBUTORS

Tseng, W.L. prepared the data, conducted the analyses, interpreted the results, and drafted the manuscript. Thomas, L.A. managed the data and provided critical feedback on the manuscript. Harkins, E. collected the data, assisted in data preparation, and provided critical feedback on the manuscript. Stoddard, J. assisted in data interpretation and provided critical feedback on the manuscript. Zarate, C.A. Jr. assisted in data collection and provided critical feedback on the manuscript. Pine, D.S. contributed to the conceptualization and design of the study and provided critical feedback on the manuscript. Leibenluft, E. contributed to the conceptualization and design of the study, data interpretation, and provided critical feedback on the manuscript. Brotman, M.A. contributed to the conceptualization and design of the study, data preparation, data interpretation, and provided critical feedback on the manuscript. All authors approved the manuscript prior to its submission.

CONFLICT OF INTEREST

The authors disclose no conflicts of interest related to this work.

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Keywords

bipolar disorder; functional neuroimaging; backward masking; functional connectivity; face emotion processing

1. INTRODUCTION

Neural dysfunction while processing face emotions represents one of the best-replicated pathophysiological findings in the literature on bipolar disorder (BD) (for reviews and meta-analyses, see Blond et al., 2012; Chen et al., 2011; Delvecchio et al., 2012; Houenou et al., 2011; Kupferschmidt and Zakzanis, 2011; Strakowski et al., 2012). This line of research, however, has focused on consciously perceived face emotions (i.e., unmasked faces presented 40 ms) across implicit (e.g., labeling gender of emotional faces) and explicit paradigms (e.g., labeling emotions of emotional faces). Few studies have probed automatic, unconscious face emotion processing using masked faces (i.e., faces presented <40 ms) in individuals with BD (Grotegerd et al., 2014; Thomas et al., 2014). Additionally, to our knowledge, no study has examined functional connectivity during this early stage of emotional processing.

Employing masked face processing paradigms is important because prolonged viewing of emotional faces, required for explicit and implicit tasks, may obscure neural dysfunction that occurs unconsciously during early, rapid processing of emotions (Monk et al., 2008; Nomura et al., 2004). Such aberrant automatic face processing may contribute to mood dysregulation and interpersonal difficulties that often occur without subjective awareness or insight in individuals with BD. Research in healthy adults and adults with anxiety and mood disorders has implicated several brain regions underlying automatic, unconscious face processing, including the amygdala, anterior cingulate cortex, inferior frontal gyrus, and occipito-temporal visual cortical regions (e.g., Killgore and Yurgelun-Todd, 2004; Lichev et al., 2015; Morris et al., 1998; Suslow et al., 2009; Whalen et al., 1998). Regarding connectivity, higher amygdala-thalamus functional connectivity was found in women with borderline personality disorder compared to healthy women (Cullen et al., 2011). A lack of coupling of metabolic activity between the orbital frontal cortex and amygdala was observed in patients with borderline personality disorder and Intermittent Explosive Disorder relative to controls (New et al., 2007). Here, we expand the literature to adults with BD by examining neural connectivity and activation during face emotion processing both above and below awareness level.

Research using consciously perceived face emotions has implicated functional abnormalities in the fronto-limbic network in BD (e.g., Brotman et al., 2014; Chen et al., 2011). However, despite consensus in the literature that BD is associated with disrupted fronto-limbic connectivity (Strakowski et al., 2012), relatively few studies have investigated functional connectivity during emotional processing. A connectivity approach examining connections between brain regions, rather than regional activations, is important to capture the dynamic, complex interactions that mediate behavior. Past studies have used psychophysiological interaction (PPI) analyses to document abnormalities in functional connectivity in BD vs.

healthy volunteers (HV) during unmasked face emotion processing. Indeed, these studies do document disrupted functional connectivity between amygdala and prefrontal cortex (PFC). Specifically, aberrant amygdala-ventromedial PFC (vmPFC) connectivity was found across both explicit (Vizueta et al., 2012) and implicit (Ladouceur et al., 2011) face paradigms, while aberrant amygdala-ventrolateral PFC (vlPFC; Foland et al., 2008; Vizueta et al., 2012) and amygdala-dorsolateral PFC (dlPFC; Vizueta et al., 2012) connectivities were found during explicit face paradigms. Disrupted amygdala-PFC connectivity has been similarly reported in patients with BD during resting state (Torrissi et al., 2013). Although a prior study investigated functional connectivity during an implicit face paradigm (i.e., gender labeling of faces; Ladouceur et al., 2011), no functional connectivity study has used *masked* faces. Thus, the current study is the first one to examine functional connectivity during early, rapid, automatic processing of masked faces in BD patients.

With regard to regional activation during *unmasked* face processing, studies also suggest PFC hypoactivation and limbic hyperactivation in BD across explicit and implicit paradigms (for reviews and meta-analyses, see Blond et al., 2012; Chen et al., 2011; Delvecchio et al., 2012; Houenou et al., 2011; Kupferschmidt and Zakzanis, 2011; Strakowski et al., 2012). However, only two studies investigated rapid, automatic processing of *masked* faces (presented for 33 ms) in BD patients, one in youth (Thomas et al., 2014) and the other in adults (Grotegerd et al., 2014). Thomas et al. (2014) reported hyperactivation in the precentral gyrus, superior temporal gyrus, and medial frontal gyrus in BD youth relative to HV across both masked and unmasked faces, but activation in the middle occipital gyrus varied with awareness level. Grotegerd et al. (2014) found that BD adults, relative to HV, exhibited amygdala hypoactivation to masked sad faces. Grotegerd et al.'s study (2014) used masked faces (no unmasked faces), only probed amygdala activation, and did not examine functional connectivity.

The goal of this study was to compare functional connectivity and regional activation in adults with BD and HV during rapid, automatic vs. explicit processing of emotional faces. Thus, we incorporated both masked and unmasked faces in a single paradigm; investigated functional connectivity, given the importance of understanding connections between brain regions; and conducted a whole-brain analysis and a region of interest (ROI) analysis on the amygdala, an important region for automatic emotional processing (Killgore et al., 2014; Rauch et al., 2000; Whalen et al., 1998). Based on previous research in BD, we hypothesized that BD patients would show disturbed functional connectivity between amygdala and PFC, particularly vlPFC (Foland et al., 2008; Vizueta et al., 2012), vmPFC (Ladouceur et al., 2011; Vizueta et al., 2012) and dlPFC (Vizueta et al., 2012), as these frontal-limbic networks are thought to subserve explicit, elaborative as well as implicit, automatic emotional processing (Phillips et al., 2008). In addition, we hypothesized that BD patients, relative to HV, would demonstrate abnormalities in amygdala, PFC, and occipito-temporal visual cortical activation (Blond et al., 2012; Chen et al., 2011; Delvecchio et al., 2012; Grotegerd et al., 2014; Houenou et al., 2011; Kupferschmidt and Zakzanis, 2011; Lichev et al., 2015; Strakowski et al., 2012; Suslow et al., 2009). The precise nature of neural dysfunction may vary with awareness level (Thomas et al., 2014) or emotions (Thomas et al., 2014). For example, BD patients, relative to HV, may show greater activation

to masked vs. unmasked faces (Thomas et al., 2014) and greater activation to angry or happy faces vs. other faces in these regions (Phillips and Vieta, 2007; Thomas et al., 2014).

2. METHODS

2.1. Participants

Fourteen adults with BD and 14 HV (Mean age = 34.64 years, $SD = 10.2$ years) participated in this Institutional Review Board-approved study at the National Institute of Mental Health (NIMH). Written informed consent was obtained from all participants. Adults with BD were recruited through advertisements to support groups, professional meetings, and psychiatrists. HV were drawn from the community through advertisements.

The Structural Clinical Interview for DSM-IV-TR Axis I Disorders–Patient Edition (SCID-I/P; $n=11$ BD and 14 HV) (First et al., 2002) or the Diagnostic Interview for Genetic Studies (DIGS; $n=3$ BD) (Nurnberger et al., 1994) was used to determine diagnostic status for patients and controls. The Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorders Version (SIGH-SAD; Williams, 1988) and the Young Mania Rating Scale (YMRS; Young et al., 1978) were used to evaluate mood state in BD patients. Sample characteristics, including mood states and comorbidities, are described in Table 1.

HV were medication-free, and had no lifetime psychiatric diagnoses and no first-degree relatives with mood disorders. Exclusion criteria for all participants included: $IQ < 70$, history of head trauma, unstable medical illness, neurological disorder, pervasive developmental disorder, active psychotic symptoms, or substance abuse/dependence within the past 3 months.

2.2. fMRI Paradigm

Participants completed an affective priming task (Tseng et al., 2016) during fMRI data acquisition. The task consisted of four runs: two for the aware condition (with unmasked faces) and two for the non-aware condition (with masked faces). The runs were interleaved, i.e., alternating between aware and non-aware conditions, and the order of the runs was counterbalanced across participants. In the *aware* condition, subjects saw a fixation cross (1250–1750ms, average 1500ms), followed by a face or blank oval (187ms), and then an abstract shape (3000ms; see Figure 1). In the *non-aware* condition, subjects saw a fixation cross (1250–1750ms, average 1500ms), followed by a face or blank oval (17ms), a scrambled face mask (170ms), and finally an abstract shape (3000ms; Figure 1). Eprime was used to present the stimuli. The stimulus onset and duration were recorded in Eprime and checked to ensure the exact timing and duration are good. The facial stimuli were taken from the NimStim set of facial expressions (Tottenham et al., 2009). The abstract shapes were taken from a pool of 101 exemplars. The shapes were not different in each run; they were randomized without repeat. Because of randomization, there should be no associations between shapes and face stimuli. Each event was 3187ms long. To test whether behavioral responding was influenced by the emotional primes and ensure participants were attending, in both the aware and non-aware conditions, participants indicated on a scale from 1 (did not

like) to 5 (liked a lot) how much they liked the abstract shape presented after the masked or unmasked face (Figure 1). The face stimuli, angry, happy, neutral, or “no face” (blank oval), were presented randomly; there were 36 trials for each stimulus under each awareness condition. The duration of the task was approximately 28 minutes. Prior to scanning, outside the scanner on a desktop computer, participants completed a practice run of 8 trials for each awareness condition, using faces not presented during scanning.

2.3. Post-Task Assessments

Immediately after the affective priming task and during structural scanning, two tasks were administered to assess whether the awareness manipulation was successful. The order of the two post-tasks was random. In both, participants saw the faces in the non-aware condition and were informed about the presence of the face in the “flash” before the shape. In one post-task, participants were asked to identify the gender of the face. We combined all participants’ accuracy data and ran one-sample *t*-tests vs. chance (50%) for each gender (male, female). In the other post-task, subjects were asked to identify the face emotion (angry, happy, or neutral). To examine if any emotion “leaked” from the mask into awareness, we conducted one-sample *t*-tests on accuracy for angry, happy, and neutral faces vs. chance (33.3%). These tests used an intentionally liberal statistical threshold since the intent was to identify any evidence of perception of the emotion on the masked faces. We also conducted *t*-tests to examine between-group differences in accuracy between BD and HV.

2.4. Image Acquisition and Preprocessing

Data were acquired on a 3T GE scanner with an eight-channel head coil. Structural images used T1-weighted axial acquisition [three-dimensional spoiled-gradient-recall acquisition in the steady state with inversion recovery prep pulse; 256×192 matrix, 128 1.2mm axial slices, 22cm field of view (FOV)] to allow normalization to standard space (Talarach and Tournoux, 1988). Functional imaging was performed axially using a multi-slice gradient echo-planar sequence (24cm FOV, 96×96 matrix, 38 contiguous 2.6mm slices, TR=2300ms, TE=25ms, voxel size=2.6×2.5×2.5mm). The phase encoding direction of the functional images was anterior-posterior; the direction of the slice acquisitions was ascending (interleaved).

fMRI data were analyzed using Analysis of Functional NeuroImages (AFNI) program (Cox, 1996). The first four volumes in each series were discarded, leaving 704 repetition times per participant. Preprocessing included despiking, slice timing correction (performed before coregistration/motion correction), coregistration, spatial smoothing (kernel full width at half maximum=6), masking, intensity scaling, and transformation into Talairach space. Repetitions with motion >1mm relative to the preceding repetition were removed from the analysis. In the 1st level of fMRI analyses, all onsets were at the beginning of the face/oval stimuli. Events are modeled at the onset of face or oval and end after the presentation of the shape (3187 ms). Regressors for each emotion in each awareness condition [Emotion (4; angry, happy, neutral, no face) × Awareness (2; aware, non-aware)], were created by convolving stimulus times with a gamma-variate hemodynamic response function. Individual-subject linear regression modeling was performed per voxel, with 8 task

regressors (Emotion×Awareness) plus 1 regressor modeling missed trials (which did not differ between BD and HV; $p>0.05$, $M=10.9$ for BD and 10.8 for HV), a third-order polynomial modeling the baseline drift, and 6 motion parameters. Beta coefficients and t -statistics were calculated for each voxel and regressor. Blank-fixation trials provided a baseline.

2.5. Data Analysis

2.5.1. Behavioral data—Ratings and reaction time (RT) on the affective priming task were compared in two separate Diagnosis (2; BD, HV) × Emotion (4; angry, happy, neutral, no face) × Awareness (2; aware, non-aware) repeated-measures analyses of variance (ANOVAs), with Emotion and Awareness as within-subject variables and Diagnosis as a between-subject variable.

2.5.2. Functional connectivity analyses—To assess context-dependent connectivity between a seed region, i.e., bilateral amygdalae, and the rest of the brain, we performed PPI analyses using a generalized PPI approach (McLaren et al., 2012). PPI analyses identify how neural activity within brain networks is modulated by the different psychological states within an fMRI task (McLaren et al., 2012). The generalized PPI approach, unlike the standard PPI approach, can accommodate more than two task conditions, and may improve single-subject model fit and the specificity and sensitivity of the analyses (Cisler et al., 2014; McLaren et al., 2012). Generalized PPI is also more flexible than standard PPI, as it allows for examinations of connectivity in individual conditions or a combination of individual conditions relative to baseline (fixations) rather than a contrast between two conditions (McLaren et al., 2012). We chose bilateral amygdalae as the seed region, given that masked faces are thought to bypass top-down cortical regulation of the amygdala and elicit amygdala responses (Killgore et al., 2014; Rauch et al., 2000; Whalen et al., 1998). For each subject, average time-series data were extracted from the seed region, i.e., left and right amygdalae defined by the Talairach-Tournoux Daemon, after removing noise (e.g., motion, baseline drift). These time series data were deconvolved with a gamma-variate hemodynamic response function before a PPI term was generated for each of the 8 task conditions [Emotion(4)×Awareness(2)] by multiplying the seed time-series data and the task condition vector (i.e., onset times plus stimulus duration for each task condition). These PPI terms were then reconvolved to create PPI regressors that were used in the individual-subject regression analysis. Regressors in the individual-subject PPI analysis included: 8 task regressors (Emotion×Awareness) plus 1 regressor modeling missed trials, seed region time series, and 8 PPI regressors. Subject-level PPI statistical images were then taken to the group level to evaluate within-group and between-group differences using a Diagnosis (2) × Emotion (4) × Awareness (2) ANOVA. The PPI analyses were conducted separately for left and right amygdalae.

3dClustSim based on Monte Carlo simulations using group blur estimates indicated that a cluster-extent threshold of $k = 63$ contiguous voxels at voxel-wise $p<0.005$ resulted in a whole-brain false positive probability of $p<0.05$. Clusters surpassing this threshold are reported. Average connectivity estimates were extracted, and follow-up ANOVAs or t -tests were performed in SPSS to decipher significant main effects or interactions. We applied

Bonferroni correction to the follow-up analyses for multiple comparisons; adjusted p values were reported. Given the recent concern regarding inflated false positive rates for cluster-threshold based fMRI statistics programs such as AFNI 3dClustSim (Eklund et al., 2016), we also conducted analyses using a modified 3dClustSim method [autocorrelation function (ACF)] that accounts for the long-tailed feature of the spatial correlation of fMRI noises (Cox et al., 2016). Based on the modified 3dClustSim with ACF, a cluster-extent threshold of $k = 97$ contiguous voxels at voxel-wise $p < 0.005$ would result in a whole-brain false positive probability of $p < 0.05$. We focused our discussion on the findings that survived this threshold. However, because 3dClustSim with ACF is a newly developed correction method, we included the findings without 3dClustSim ACF correction in the results, tables, and figures, for comparisons with the existing literature.

2.5.3. Whole-brain activation pattern—At the group level, we conducted a whole-brain Diagnosis (2) $\times$ Emotion (4) $\times$ Awareness (2) ANOVA. There were no suprathreshold voxels in this three-way ANOVA. Given our primary interest in differences between patients and controls, analyses then focused on the two-way ANOVAs that included diagnosis, i.e., Diagnosis $\times$ Emotion and Diagnosis $\times$ Awareness. Findings that survived a cluster-extent threshold of $k = 63$ and voxel-wise $p < 0.005$ (whole-brain corrected $p < 0.05$) are reported; however, our discussion focused on findings that survived the new threshold with 3dClustSim ACF ($k = 97$ and voxel-wise $p < 0.005$). Average signal change values were extracted, and follow-up ANOVAs or t -tests were performed in SPSS to understand significant main effects or interactions. We applied Bonferroni correction to these follow-up analyses for multiple comparisons; adjusted p values were reported.

2.5.4. ROI analyses—Because masked faces are thought to elicit amygdala responses (Killgore et al., 2014; Rauch et al., 2000; Whalen et al., 1998), we also conducted ROI analyses on the left and right amygdalae defined by the Talairach-Tournoux Daemon. Mean signal intensity was extracted from each ROI for each stimulus type and was submitted to a Diagnosis (2) $\times$ Emotion (4) $\times$ Awareness (2) ANOVA in SPSS.

2.5.5. Post-hoc analyses—The average connectivity estimates and the average signal change were extracted from significant clusters in the main analyses and used in the post-hoc analyses. Given trend-level between-group differences in age, post-hoc analyses in SPSS were conducted to examine its effect on the imaging findings. We also conducted post-hoc analyses in SPSS to test the effects of RT, medication, mood symptoms, mood state (euthymic vs. depressed), and anxiety comorbidity on the functional connectivity and brain activation in BD patients.

3. RESULTS

3.1. Demographics

Chi-square analysis or t -tests were used to compare gender distribution, age, and IQ between groups. There was a numerical between-group difference in age ($p < 0.10$), i.e., BD patients were slightly older than HV. Post-hoc analyses were conducted to examine the effect of age on our imaging findings (see below).

3.2. Behavioral Data

3.2.1. Rating—There were no significant three-way (i.e., Diagnosis $\times$ Emotion $\times$ Awareness) or two-way (i.e., Diagnosis $\times$ Emotion, Diagnosis $\times$ Awareness, or Emotion $\times$ Awareness) interactions, and no main effect of Diagnosis. Main effects of Emotion ($F_{3,78}=3.56$, $p<0.05$, $\eta_p^2=0.12$) and Awareness ($F_{1,26}=4.84$, $p<0.05$, $\eta_p^2=0.16$) were significant. Participants liked shapes presented after angry faces more than those presented after happy faces ($p<0.05$, $d=0.41$). Participants liked shapes presented in the non-aware condition more than those in the aware condition ($p<0.05$, $d=0.29$).

3.2.2. RT—Similarly, there were no significant three-way or two-way interactions, or main effects of Diagnosis and Awareness. The main effect of Emotion was significant ($F_{3,78}=12.57$, $p<0.001$, $\eta_p^2=0.33$), i.e., participants responded to shapes more slowly after angry or neutral faces than after no face ($ps<0.01$, $ds=0.20$ and 0.26); participants also responded more slowly after neutral faces than after happy faces ($p<0.01$, $d=0.18$).

3.2.3. Post-Tasks—Data from the two post-tasks indicated that participants were unaware of the emotional face prime. On both the post-task gender and emotion identification tasks, accuracy was no better than chance level for any emotion (i.e., $M=46.6$ – 50.7% for gender identification, $ps>0.05$; $M=27.0$ – 36.7% for emotion identification, $ps>0.05$), and BD and HV did not differ across face emotions ($ps>0.05$).

3.3. fMRI Data

3.3.1. Functional Connectivity—PPI analyses using left amygdala as the seed revealed a Diagnosis $\times$ Awareness interaction in the left vIPFC (Table 2, Figure 2); that is, the magnitude of functional connectivity between left amygdala and left vIPFC varied as a function of diagnosis and awareness condition. Within BD patients, connectivity was greater in the aware than in the non-aware condition ($p<0.05$, $d=0.39$); within HV, connectivity was greater in the non-aware than in the aware condition ($p<0.001$, $d=-1.07$; Figure 2). Relative to HV, BD patients showed *diminished* functional connectivity during the non-aware condition (at a trend level, $p<0.10$, $d=0.63$) but *greater* connectivity during the aware condition (at a trend level, $p<0.10$, $d=-0.74$; Figure 2). When restricting the analyses to faces only (i.e., angry, happy, neutral faces), we found a similar pattern of findings. That is, within BD patients, connectivity was greater in the aware than in the non-aware condition ($p<0.01$, $d=0.39$); within HV, connectivity was greater in the non-aware than in the aware condition ($p<0.05$, $d=-0.89$). In addition, relative to HV, BD patients showed *diminished* functional connectivity during the non-aware condition ($p<0.05$, $d=0.84$) but did not differ from HV during the aware condition ($p>0.10$, $d=-0.40$).

PPI analyses also revealed a main effect of Diagnosis in the left vmPFC (Table 2, Figure 3). The main effect of Diagnosis represents the group differences in connectivity across the entire task (across all conditions and emotions) while subjects were viewing faces/masks/shapes vs. baseline (fixations). BD patients, relative to HV, exhibited diminished functional connectivity between left amygdala and left vmPFC across the task ($p<0.001$, $d=1.61$; Figure 3). No significant interactions or main effects emerged from PPI analyses with right amygdala as the seed region.

3.3.2. Whole-brain activation pattern—There were no significant findings from the Diagnosis $\times$ Emotion $\times$ Awareness interaction. Given our interest in between-group differences, we next examined the two-way interactions of Diagnosis $\times$ Emotion and Diagnosis $\times$ Awareness.

A Diagnosis $\times$ Emotion interaction was present in the right medial frontal gyrus/anterior cingulate cortex, $x=11$, $y=31$, $z=29$, voxel-wise $p=0.005$, $k=360$ (Table S1 in the supplementary materials, Figure 4). Follow-up analyses indicated significant within-group differences (i.e., main effect of Emotion) in BD ($F_{3,39}=5.21$, $p<0.01$, $\eta_p^2=0.29$) and HV ($F_{3,39}=5.38$, $p<0.01$, $\eta_p^2=0.29$). Specifically, BD patients displayed *greater* activation to angry and neutral faces compared to no face ($ps<0.05$, $ds=0.57$). HV, in contrast, displayed *reduced* activation to angry and neutral faces compared to no face ($ps<0.05$, $ds=0.43$ and 0.55). Between-group differences in each emotion type in this region were not significant.

A Diagnosis $\times$ Emotion interaction was also present in the right precuneus, $x=26$, $y=-54$, $z=36$, voxel-wise $p=0.005$, $k=64$ (Table S1 in the supplementary materials, Figure 5). Follow-up analyses indicated significant within-group differences (i.e., main effect of Emotion) in BD only ($F_{3,39}=11.18$, $p<0.001$, $\eta_p^2=0.46$). BD patients displayed *more* activation to angry faces compared to happy faces and no face ($ps<0.05$, $ds=0.45$ and 0.59). There were also significant between-group differences in this region. That is, BD patients, relative to HV, showed *more* activation in precuneus across emotion types ($ps<0.05$, $ds=0.97-1.56$).

When restricting these analyses to masked and unmasked stimuli separately, the patterns of within-group and between-group findings were similar, suggesting that the effects were across masked and unmasked stimuli and not specific to either type of stimuli.

No significant clusters were found for Diagnosis $\times$ Awareness interaction.

3.3.3. ROI analyses—In the bilateral amygdalae, there were no significant three-way or two-way interactions or main effects of Diagnosis, Emotion, and Awareness ($ps>0.05$).

3.4. Post-hoc Analyses

Both the PPI and whole-brain activation results remained significant when covarying age and RT ($ps<0.01$). There were no associations between functional connectivity or brain activation and number of medications, YMRS scores, or SIGH-SAD scores in BD patients ($ps>0.05$). When comparing euthymic BD patients ($n=8$) to depressed BD patients ($n=5$), the two groups did not differ in functional connectivity ($ps>0.05$) or brain activation. When comparing BD patients with ($n=7$) vs. without ($n=7$) anxiety disorders, the two groups did not differ in functional connectivity or brain activation (all $ps>0.05$).

4. DISCUSSION

The goal of this study was to examine neural connectivity and correlates of face emotion processing above and below awareness level in adults with BD. This is the first study to assess functional connectivity during early, rapid, automatic emotional processing of masked

faces in BD patients. Our data indicated aberrant amygdala-vmPFC connectivity in BD irrespective of awareness level and emotion type. In addition, we found functional abnormalities in the medial frontal gyrus/anterior cingulate cortex in BD. Behaviorally, BD patients and HV did not differ. However, consistent with previous research, participants rated shapes presented after angry faces more favorably than those presented after happy faces (Thomas et al., 2014; Tseng et al., 2016); participants responded to shapes more slowly after angry or neutral faces were presented than after no face was presented; participants also responded more slowly after neutral faces than after happy faces (Tseng et al., 2016).

Across the entire task of masked and unmasked processing, BD patients, relative to HV, exhibited diminished functional connectivity between amygdala and vmPFC. Aberrant amygdala-vmPFC connectivity during an implicit (gender labeling; Ladouceur et al., 2011) and an explicit face processing paradigm (face-matching; Vizueta et al., 2012) has also been implicated in the past research in BD. This is somewhat consistent with our finding across task condition, although implicit paradigms such as gender labeling are not equivalent to masked face processing paradigms. The vmPFC has robust connections to the amygdala, both structurally and functionally (Banks et al., 2007; Kim et al., 2011; Ghashghaei and Barbas, 2002), and is important in generating affective meaning (Roy et al., 2012), regulating limbic responses to emotional stimuli (Etkin et al., 2011), and flexibly updating signal value (Viviani, 2014). Thus, perturbed amygdala-vmPFC connectivity may mediate over-reactivity to face stimuli, regardless of the awareness level.

Results from the whole-brain activation analyses indicated that BD patients showed *greater* activation to angry and neutral faces compared to no face in the medial frontal gyrus/anterior cingulate cortex, whereas HV showed *reduced* activation in this region. This was true across both masked and unmasked faces. The dorsal-caudal region of the medial frontal gyrus and anterior cingulate cortex has been implicated in appraisal and expression of emotions (Etkin et al., 2011; Shackman et al., 2011). Greater activation in this area was reported in healthy individuals with high emotional awareness relative to those with low emotional awareness during masked processing of happy faces (Lichev et al., 2015). It is argued that positive mood state of healthy participants may induce mood-congruent response biases favoring the processing of happy faces (Lichev et al., 2015). It is possible that BD patients may show mood-congruent *negative* affective priming effects, and thus enhanced activation to faces of negative valences, consistent with findings in other clinical populations such as depressed and schizophrenia patients (Dannlowski et al., 2006; Suslow et al., 2003).

The data also showed that BD patients exhibited *reduced* amygdala-vlPFC connectivity when viewing masked vs. unmasked faces, while HV showed the opposite pattern; BD patients, relative to HV, exhibited hyperactivation in precuneus across emotion types. Although these findings did not surpass the whole-brain correction threshold using the new 3dClustSim ACF method, they are informative. Balancing Type I and Type II errors is an important issue, especially in clinical fMRI studies (Carter, Lesh, & Barch, 2016). While some prioritize concerns regarding false-positive results (Eklund et al., 2016; Woo et al., 2014), clinical imaging studies, such as the current one, also concern false-negative results. Thus, a high voxel or cluster-extent threshold may not be the most appropriate in clinical imaging studies (Carter, Lesh, & Barch, 2016). Because of this and the limited literature on

masked face processing in BD, we retained these findings in the paper to stimulate hypothesis generations for future research in this understudied area.

Unlike previous research on unmasked (for reviews, see Blond et al., 2012; Chen et al., 2011; Delvecchio et al., 2012; Houenou et al., 2011; Kupferschmidt and Zakzanis, 2011; Strakowski et al., 2012) and masked (Grotegerd et al., 2014) face processing in BD, this study did not find between-group differences in amygdala activation. It should be noted that in a similar paradigm, Thomas et al. (2014) also did not find amygdala dysfunction in pediatric BD. This null finding could be attributed to several factors, including Type II error and discrepancies in paradigms across studies. For example, the only adult BD study with *masked* faces (Grotegerd et al., 2014) reported amygdala hypoactivation in depressed BD patients, relative to HV, but only in sad faces, which were not used in the current study. Furthermore, in previous studies, face emotions were displayed for a longer duration (33ms) than in the present study (17ms for masked faces). Thus, the involvement of amygdala, particularly in masked face processing, in BD remains to be determined pending future work with a larger sample.

Our findings should be interpreted in light of the following limitations. First, the sample was relatively small and heterogeneous. This may have limited the power of our analyses to detect significant Diagnosis $\times$ Emotion $\times$ Awareness interactions. Future research with a larger and more homogenous sample is warranted to replicate our findings. Furthermore, as is typical in fMRI studies of patients with BD, more than half had comorbid disorders (54.5%), the majority were medicated (85.7%), and the sample included both depressed and euthymic subjects. Although our post-hoc analyses suggested that current comorbidities, medications, and mood state did not contribute to our findings, these analyses may have been underpowered. Thus, the effects of these confounding variables on our findings are uncertain. In addition, two adult BD patients had a history of substance abuse or dependency, albeit not within the past 3 months. We conducted sensitivity analyses, removing these two patients, and found that the significant results still held. This suggests that our findings may not be driven by substance abuse/dependency history. Again, these analyses may be underpowered; it is unclear the extent to which substance abuse/dependency history contributed to our findings.

Despite these limitations, this study provides the first data on functional connectivity abnormalities in BD during rapid, automatic emotional processing. Aberrant amygdala-vmPFC connectivity along with neural dysfunction in areas implicated in appraisal and expression of emotions (medial frontal gyrus) may be the pathophysiological correlates of emotional processing in BD regardless of awareness level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Little is known regarding the neural connectivity and correlates during automatic, unconscious face emotion processing in individuals with bipolar disorder (BD).
- Aberrant amygdala-ventromedial prefrontal cortex connectivity along with neural dysfunction in areas implicated in appraisal and expression of emotions (medial frontal gyrus) may be the pathophysiological correlates of emotional processing in BD regardless of awareness level.

Figure 1.
Affective priming paradigm. (A) Non-aware condition. (B) Aware condition.

Figure 2.

Diagnosis $\times$ Awareness interaction from the whole-brain analysis of variance (ANOVA) of functional connectivity between left amygdala and left ventrolateral prefrontal cortex (vlPFC). *Note.* HV = Healthy Volunteers, BD = Bipolar Disorder. Cluster survived whole-brain correction at $p < .05$ using 3dClustSim without autocorrelation function. † $p < 0.10$, * $p < 0.05$, *** $p < 0.001$

Figure 3. Diagnosis main effect from the whole-brain ANOVA of functional connectivity between left amygdala and left ventromedial prefrontal cortex (vmPFC). *Note.* HV = Healthy Volunteers, BD = Bipolar Disorder. Cluster survived whole-brain correction at $p < .05$ using 3dClustSim without autocorrelation function. *** $p < 0.001$

Figure 4. Diagnosis $\times$ Emotion interaction in the right medial frontal gyrus/anterior cingulate cortex from the whole-brain ANOVA of activation. *Note.* HV = Healthy Volunteers, BD = Bipolar Disorder. Cluster survived whole-brain correction at $p < .05$ using 3dClustSim without autocorrelation function. * $p < 0.05$

Figure 5. Diagnosis $\times$ Emotion interaction in the right precuneus from the whole-brain ANOVA of activation. *Note.* HV = Healthy Volunteers, BD = Bipolar Disorder. Cluster survived whole-brain correction at $p < .05$ using 3dClustSim without autocorrelation function. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 1

Sample Characteristics

Characteristic	BD (<i>n</i> = 14)	HV (<i>n</i> = 14)	<i>t</i> or χ^2	<i>p</i> value
	Mean + SD	Mean + SD		
Age (years)	37.94 ± 9.98	31.34 ± 9.66	1.78	<0.10
Full-scale IQ	114.86 ± 9.53	121.14 ± 13.28	−1.44	>0.05
YMRS ^a	4.00 ± 3.19	--	--	--
SIGH-SAD ^a	16.08 ± 9.37	--	--	--
Number of Medications	3.43 ± 1.83	--	--	--
	N (%)	N (%)		
Male	3 (21.4)	3 (21.4)	0.00	>0.05
Bipolar Type				
Bipolar I	9 (64.3)	--	--	--
Bipolar II	5 (35.7)	--	--	--
Mood State ^{a,b}				
Euthymic	8 (61.5)	--	--	--
Depressed	5 (38.5)	--	--	--
Hypo/manic	0 (0)	--	--	--
Mixed	0 (0)	--	--	--
Comorbid Conditions				
Any Comorbidity	7 (50.0)	--	--	--
ADHD	0 (0)	--	--	--
Eating Disorder	1 (7.1)	--	--	--
Any Anxiety Disorder ^c	7 (50.0)	--	--	--
Past history of substance abuse/dependency	2 (14.3)	--	--	--
Medication at scan				
Unmedicated	2 (14.3)	14 (100)	--	--
Atypical Antipsychotic	9 (64.3)	0	--	--
Lithium	7 (50.0)	0	--	--
Antiepileptic	9 (64.3)	0	--	--
Antidepressant	7 (50.0)	0	--	--
Stimulants	1 (7.1)	0	--	--

Note. BD = Bipolar Disorder, HV = Healthy Volunteers, YMRS = Young Mania Rating Scale, SIGH-SAD = Structural Clinical Interview for the Hamilton Depression Scale–Seasonal Affective Disorder Version, ADHD = Attention-Deficit Hyperactivity Disorder, CD = Conduct Disorder.

^a Missing data for 1 BD. For categorical variables, percentages are calculated based on *n*=13, instead of *n*=14.

^b Euthymic was defined as YMRS ≤ 12 and SIGH-SAD ≤ 20; depressed was defined as YMRS > 12 and SIGH-SAD > 20; hypo/manic was defined as YMRS > 12 and SIGH-SAD > 20; mixed state was defined as YMRS > 12 and SIGH-SAD > 20.

^c Includes generalized anxiety disorder, specific phobia, social phobia, panic disorder, post-traumatic stress disorder, and obsessive compulsive disorder.

Table 2

Functional Connectivity between left amygdala and other brain regions using generalized psychophysiological interaction (PPI) analyses.

Area of co-activation	Brodmann area (BA)	Side	Cluster Size	3dClustSim corrected <i>p</i> value ^a	3dClustSim ACF corrected <i>p</i> value ^b	Talairach Coordinates ^c			Analysis ^d	
						x	y	z	<i>F</i> (1, 26)	<i>p</i>
Diagnosis × Awareness vIPFC	BA 44/45	Left	70	<0.04	>0.10	-51	16	14	23.74	<0.001
Diagnosis vmpFC	BA 10/32	Left	100	<0.01	<0.05	-1	46	-6	18.24	<0.001
										η_p^2
										0.48
										0.41

Note. vIPFC = ventrolateral prefrontal cortex, vmpFC = ventromedial prefrontal cortex.

^aWhole-brain corrected *p* values based on 3dClustSim without autocorrelation function.

^bWhole-brain corrected *p* values based on 3dClustSim autocorrelation function (ACF).

^cCoordinates refer to the voxel with maximum signal intensity (i.e., peak value).

^dStatistics refer to the analysis of the extracted clusters in SPSS.