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## Intrinsic Amygdala Functional Connectivity in Youth With Bipolar I Disorder

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### Abstract

**Objective**—Bipolar disorder (BD) commonly begins during adolescence and may continue into adulthood. Studies in adults with BD suggest that disruptions in amygdalar neural circuitry explain the pathophysiology underlying the disorder. Importantly, however, amygdala subregion networks have not yet been examined in youth close to mania onset. The goal of this study was to compare resting state functional connectivity patterns in amygdala subregions in youth with bipolar-I (BP-I) disorder with patterns in healthy controls.

**Method**—Centromedial, laterobasal, and superficial amygdala subdivisions were assessed during rest and examined in relation to clinical measures of mania in youth (14 to 20 years old) with BP-I who experienced only a single episode of mania (n=20, “BD”) and age-matched healthy comparison youth without any personal or family history of *DSM-IV* Axis I disorders (n=23, “HC”).

**Results**—Relative to HC youth, youth with BD exhibited decreased connectivity between the laterobasal subdivision of the amygdala and the hippocampus and precentral gyrus, and increased connectivity between the laterobasal subdivision and the precuneus. Connectivity between the right laterobasal amygdala and right hippocampus was positively correlated with levels of anxiety in BD but not in HC youth, and connectivity between the right laterobasal amygdala and right precuneus was negatively correlated with insight about bipolar illness.

**Conclusion**—Youth with BD have abnormal amygdala resting state network connections to regions that are critical for emotional processing and self-awareness. Longitudinal studies are needed to determine whether these aberrant patterns in youth with BD can be altered with intervention and influence the course of disorder.

### Keywords

bipolar; resting state functional connectivity; amygdala; precuneus; hippocampus

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## INTRODUCTION

Bipolar disorder (BD) is a complex psychiatric disorder characterized by disturbances in cognitive functions that are subserved by distributed neural networks.<sup>1,2,3</sup> Childhood onset of BD is common and is associated with a more severe course and prognosis than is onset occurring during adulthood;<sup>4,5</sup> thus, efforts to identify neurobiological factors associated with early onset and progression of BD are particularly important. Several lines of evidence indicate that BD is associated with impairments in key cognitive processes critical for emotional processing,<sup>3,6,7</sup> and by anomalies in both neural structure and function, particularly in the amygdala, hippocampus, ventrolateral (VLPFC) and dorsolateral (DLPFC) prefrontal cortex, striatum, and portions of the anterior cingulate cortex (ACC).<sup>8</sup> These findings raise the possibility that BD arises from a disruption of connections between specific prefrontal-limbic networks. In particular, investigators have documented that, compared to adult-onset BD, childhood-onset BD is uniquely characterized by reductions in amygdala volume<sup>9,10</sup> and amygdala hyperactivity,<sup>11-14</sup> which may represent neural markers of illness onset. The intrinsic connectivity of the amygdala, however, has not been examined in youth with BD close to the onset of manic illness, and may be critical to clarifying a network-based neurodevelopmental model of BD.<sup>15</sup>

Recent studies have used resting-state functional magnetic resonance imaging (fMRI) to characterize networks associated with BD. This procedure circumvents task-related confounds such as performance variance and probes ongoing spontaneous brain activity that provides a rich potential source of disorder-related signal change.<sup>16</sup> Adults with BD have been found to exhibit decreased resting-state connectivity between the pregenual ACC and amygdala, thalamus, and pallidostriatum,<sup>17</sup> and loss of inverse connectivity between VLPFC and amygdala.<sup>18</sup> Thus, disrupted functional connectivity in key limbic and prefrontal regions may underlie the core deficits in emotional processing associated with BD.

Several studies have now used a variety of regionally specific and model-free analytic approaches to compare resting state functional connectivity between youth with BD and healthy controls. Two studies used a region of interest (ROI) analysis approach: one reported increased connectivity between the left DLPFC and superior temporal cortex in youth with BD relative to healthy youth,<sup>19</sup> and the other reported decreased amygdala-posterior insula connectivity in BD youth.<sup>20</sup> Using a model-free independent components analysis (ICA) approach, Wu et al.<sup>21</sup> found that youth with BD exhibited altered affective, executive, and sensorimotor networks, and that greater connectivity of the right amygdala within the affective network was associated with better executive function in children with BD, but not in controls. Collectively, the results of these studies suggest that pediatric BD is characterized by aberrant patterns of prefrontal and limbic connectivity.

It is difficult to draw strong conclusions and develop a clear pathophysiological model of pediatric BD from these studies, however, because they used analytic approaches that are not easily compared (e.g., regionally specific versus model-free). These investigations were also limited because they combined heterogeneous samples of children and adolescents with BD without consideration of the contributions of puberty or mood state to neural findings.<sup>22</sup> These limitations highlight methodological and sampling issues that may influence reported

findings and reduce the replicability of results across studies. Most importantly, none of these studies considered the possibility that key prefrontal-limbic regions implicated in BD have subregions with structurally and functionally distinct nuclei that interact differentially with other brain networks. Further, the most consistent brain structural<sup>9</sup> and functional<sup>11–13</sup> findings specific to pediatric BD are found in the amygdala. Importantly, however, the amygdala is not homogeneous; it has subregions with structurally and functionally distinct nuclei that have different patterns of connectivity with prefrontal and subcortical networks during the processing of emotional material.<sup>23</sup> Further, researchers have documented changes in amygdala subregion-cortical functional connectivity from childhood to adolescence<sup>24</sup> that may represent a hierarchical integration of functions across subregions that has implications for the onset of BD during this developmental period.<sup>25</sup> Thus, examining connectivity in amygdala subregions has the potential to significantly advance our understanding of specific patterns of functional connectivity that are implicated in the pathophysiology of BD.

In this study we examined intrinsic amygdala subregion functional connectivity in post-pubertal youth diagnosed with bipolar I (BP-I) disorder, along key symptom dimensions, within a year of their initial manic episode. Drawing on fMRI findings in the amygdala obtained during rest in adults and youth with BD,<sup>17,18,20,21</sup> we predicted that, compared with healthy youth, youth with BP-I would exhibit disrupted functional connectivity at rest between amygdala subregions and key brain regions associated with emotion expression and emotion regulation, including the VLPFC, ACC, and insula. We also predicted that these aberrant patterns of connectivity in amygdala subregions would be associated with core symptom dimensions of BD.

## METHOD

### Participants

The university's panel of medical research in human participants approved this research protocol. After hearing a complete description of the study, parents and youth under the age of 18 years gave written informed consent and assent, respectively; youth 18 years and older gave written informed consent. Youth (ages 13-21 years) with BP-I (n=20) were recruited either by referral to a pediatric bipolar disorders clinic or from the surrounding community. HC youth (n=23) without any personal or family history of psychiatric diagnoses or psychotropic medication exposure were recruited through local community advertisements. A telephone screening with a parent established that all participants were fluent in English and did not have any metal in their body, history of head injury (with loss of consciousness over 5 minutes), seizures, or developmental disorders. Youth with BD who were prescribed stimulants did not take them 24 hours prior to neuroimaging and were required not to have used recreational drugs for at least 30 days prior to the MRI scan. To avoid risk of mood destabilization, participants with BD were allowed to continue any other psychotropic medications, including lithium, atypical antipsychotics, anticonvulsants (including valproate, lamotrigine, carbamazepine, or topiramate), and antidepressants.

## Assessment of Psychopathology

All participants were administered semi-structured clinical interviews to evaluate the presence of past or current psychiatric disorder. Trained interviewers with established symptom and diagnostic inter-rater reliability ( $\kappa > 0.9$ ) assessed the diagnostic status of all youth by administering the Affective Modules of the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U KSADS)<sup>26</sup> and the Kiddie Schedule of Affective Disorders and Schizophrenia Present and Lifetime version (KSADS-PL).<sup>27</sup> Interviewers had previous training and experience with this interview and administered it separately to the youth and their parents (about the youth) in order to assess current and lifetime psychiatric diagnoses. Diagnostic decisions were ultimately made by a board-certified child psychiatrist (M.S.) and were based on personal interview or discussion with a masters-level research assistant.

Youth in the group with BD were eligible to participate in the study if they met lifetime diagnostic criteria for BP-I according to both the parent and child WASH-U KSADS and KSADS-PL. A manic episode was defined by Diagnostic and Statistical Manual-4th edition-Text Revision (*DSM-IV-TR*) criteria that lasted at least one week and could not have been precipitated by exposure to recreational drugs, antidepressants, psychostimulants, or other medications or medical conditions. Youth in the HC group were eligible to participate if (a) they did not meet criteria for any past or current Axis I disorder based on both the parent and child KSADS-PL; (b) their parents did not meet criteria for any past or current Axis I disorder by Structured Clinical Interview for *DSM* Disorders; and (c) their first- or second-degree relatives did not meet criteria for an Axis I disorder by Family History Research Diagnostic Criteria.<sup>28</sup>

Symptom severity was assessed on the day of scan using the Young Mania Rating Scale (YMRS)<sup>29</sup> and the Children's Depressive Rating Scale-Revised (CDRS-R)<sup>30</sup> by raters with established reliabilities (all intra-rater intra-class correlation coefficients [ICCs]  $> 0.9$ ). Core symptoms of mania such as level of disorder insight were derived from specific items in the YMRS and correlated with pertinent imaging findings. Levels of anxiety were assessed by administering the Multidimensional Anxiety Scale for Children (MASC)<sup>31</sup> to the parents. Global functioning was determined using the Children's Global Assessment Scale (CGAS).<sup>32</sup> Levels of impulsivity were assessed by administering the Barratt Impulsiveness Scale (BIS-11) to the youth, which yielded subscale scores on the dimensions of attentional, motor (acting impetuously), and non-planning (absence of weighing upon long-term consequences of actions) trait impulsivity.<sup>33</sup> Age, sex, socioeconomic status (Hollingshead Four Factor Index),<sup>34</sup> pubertal stage (Pubertal Development Scale),<sup>35</sup> IQ (Wechsler Abbreviated Scale of Intelligence; WASI),<sup>36</sup> and handedness (Crovitz Handedness Questionnaire)<sup>37</sup> were also assessed. All demographic and clinical variables were assessed within a week of neuroimaging.

## Assessment of Memory for Emotional Words

Youth in the BD and HC groups completed the self-referent encoding task (SRET) within a week of the scan to assess endorsement of positive and negative adjectives and memory for valenced stimuli, as described previously.<sup>38</sup> Briefly, all participants sat before a computer

screen on which the words “Describes me?” were displayed, followed by one positive or negative stimulus word. Participants indicated “yes” or “no” for each word by using assigned keys on the computer keyboard, and the process was repeated for 40 stimulus words: 20 positive and 20 negative adjectives that were rated on a seven-point Likert scale. These adjectives had received a mean rating above 4.0 on one dimension (e.g., positive) and below 2.0 on the other dimension (e.g., negative). Examples of positive adjectives included “friendly,” “helpful,” “lucky,” “nice,” “winner,” and examples of negative adjectives included “angry,” “lazy,” “lonely,” “strange,” “bad.” Following the SRET, participants completed the Digit Span task from the Wechsler Adult Intelligence Scale-III (WAIS-III; Wechsler, 1997) to distract their attention from the adjectives they had just seen. Finally, participants completed an incidental recall task, in which they were given 3 minutes to recall as many of the SRET adjectives as they could, regardless of whether they had endorsed the words as self-referent.

### MRI Data Acquisition and Preprocessing

Magnetic resonance images were collected at Lucas Center of Radiology at Stanford University using a 3T GE Discovery MR750 scanner (General Electric, Milwaukee) with an 8 channel whole head coil; T2-weighted spiral in/out pulse sequence: TR=2000 msec; TE=30 msec; flip angle 80°; field of view 220 mm × 220 mm, voxel-size 3.43 mm × 3.43 mm × 4 mm with 1 mm skip, 30 slices collected in ascending order, anterior and posterior commissure alignment, 210 volumes, and high-order shimming. Participants were scanned for 7 minutes while instructed to remain awake but resting quietly with their eyes closed. Three-dimensional high-resolution T1-weighted anatomical images were acquired using a spoiled gradient recall (SPGR) pulse sequence with the following parameters: repetition time (TR)=6.4 msec, echo time (TE)= 2 msec, inversion time (TI)=300msec, number of excitation (NEX) scans=3, flip angle 15°, 124 coronal slices, 1.5 mm slice thickness, field of view (FOV) = 220 mm × 220 mm, 256 × 256 matrix.

Image preprocessing was performed using the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL version 5.0.4; [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). The following preprocessing steps were applied to the functional data: (a) first six volumes were discarded to allow for signal stabilization; (b) head motion correction was performed using the Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT)<sup>39</sup>; (c) non-brain tissue was extracted using the Brain Extraction Tool (BET)<sup>40</sup>; (d) spatial smoothing was conducted using a Gaussian kernel of 6-mm full width half maximum; (e) high-pass temporal filtering (Gaussian-weighted least mean squares straight line fitting with sigma = 75 seconds or 0.006 Hz) was applied to the data; and (f) low-pass temporal filtering (half width at half maximum of 2.8 seconds or 0.17 Hz) was applied to the data. After preprocessing, the functional data were registered to each individual's high-resolution T1-weighted image, followed by registration to the MNI152 standard-space by affine linear registration using FMRIB's Linear Image Registration Tool (FLIRT).<sup>39</sup>

Several sources of noise were regressed from the functional data, including six motion parameters and time series extracted from the white matter and cerebrospinal fluid. Importantly, the BD and HC groups did not differ on any of the six motion parameters (x;

$p=.08$ ,  $y$ ;  $p=.59$ ,  $z$ ;  $p=.11$ , pitch;  $p=.44$ , roll;  $p=.90$ , yaw;  $p=.12$ ). A check for excessive motion was conducted based on the limit of 2mm maximum translation and a limit of  $2^\circ$  maximum rotation; no participants were excluded based on these limits. Mean absolute displacement measurements were not significantly different between groups (BD:  $0.23\pm.21$ , HC:  $0.23\pm.24$ ;  $t=.12$ ,  $p=.91$ ).

Recent work has shown that in addition to regressing out motion parameters and testing for maximum translation or rotation, it is important to detect and remove individual motion-affected volumes to avoid spurious, but systematic, connectivity results<sup>41,42</sup>. Thus, volumes affected with excessive or sharp motion were detected using the *fsl\_motion\_outliers* script (supplied with FSL) and were regressed out as confounding variables. This alternative approach of regressing affected volumes as opposed to simply removing them is better as it adjusts for the changes in signal and auto-correlation on either side of the affected volume and also appropriately corrects for the degrees of freedom. Finally, the residual image was normalized for further analysis.

### Amygdala Seed-Based Connectivity

Left and right amygdala subregion ROIs (centromedial [CM], laterobasal [LB], and superficial [SF]) were determined using stereotaxic, probabilistic maps of cytoarchitectonic boundaries developed by Amunts et al.<sup>43</sup> and implemented in FSL's Juelich histological atlas (Figure 1). These ROIs included voxels exceeding 50% probability of belonging to that subregion; overlapping voxels were allocated to the ROI with the highest probability. Based on this atlas, the LB subregion encompasses the basomedial, basolateral, paralaminar, and lateral nuclei, the CM subregion encompasses the medial and central nuclei, and the SF subregion encompasses the anterior amygdaloid area, the amygdalopyriform transition area, the amygdaloid-hippocampal area, and the ventral and posterior cortical nuclei.<sup>23</sup>

Primary eigenvariates from each ROI were extracted from residual images created after noise regression. Using eigenvariate time series instead of mean value across ROI allows for better represented signal dynamics by characterizing the dominant observed signal variation within the ROI. In addition, this approach minimizes the risk of confounding the time series with signal variation from misspecified tissue due to imprecise mask boundaries between adjacent ROIs.<sup>44</sup> Nevertheless, the use of eigenvariates does not rule out the possibility of misspecified tissue in amygdala subregion ROIs, which we mitigated by careful quality-control procedures (i.e. co-registration of the functional data to the individual's SPGR prior to normalizing to the MNI152 template, and visual inspection of amygdala placement for SPGR co-registration and template registration to ensure accuracy). The *fslmeans* script was used to extract eigenvariates from each ROI. All subregion eigenvariate time series were modeled together in a multiple regression analysis (using the FMRI Expert Analysis Tool [FEAT]) to estimate the ROI connectivity maps for each participant.

Subregion connectivity estimates generated from each participant were entered into a group-level mixed-effects analysis using FSL's Flame-1 procedure with a threshold of  $p<.05$ , cluster-corrected (at  $Z=2.3$ ) to control for family-wise error (FWE). Mean and peak connectivity estimates for each participant were extracted from significant between-group clusters.

## Relations Between Functional Connectivity and Clinical Variables

We conducted univariate analyses to determine the distributions of demographic and clinical data. We extracted connectivity estimates from clusters in which there were group differences in the connectivity analyses in order to examine correlates of dysfunctional connectivity following the clinical onset of mania. We computed correlations between connectivity estimates and characteristics of youth, including age and measures of mania, depression, and trait impulsivity within each group. We also explored relations between connectivity estimates and key symptom dimensions (anxiety, insight) and cognitive (memory) functions within each group. Because these clinical correlates have not commonly been investigated in youth with BD, these analyses were conducted with the intent of being hypothesis-generating, and require replication with larger samples. Given this intent and context, we did not correct for multiple comparisons. We conducted Fisher's  $r$  to  $z$  transformations to determine whether these correlations differed significantly between the BD and HC groups. Finally, when there were sufficiently large subsamples, we explored the effects of medication exposure on amygdala subregion connectivity. We did not control for medication exposure in our primary analyses because the majority of youth in the group with BD were taking at least one medication, and combining medications that have different neural targets (and thus different mechanisms of action) would limit our ability to accurately interpret findings from the primary analyses.

## RESULTS

### Participants

Demographic and clinical variables are presented in Table 1. BD and HC groups were balanced for gender ( $\chi^2=.151$ ,  $p=.79$ ) and handedness ( $\chi^2=.022$ ,  $p=.88$ ). There were no significant group differences in age ( $t[41]=-0.69$ ,  $p=.49$ ), WASI scores ( $t[41]=1.28$ ,  $p=.21$ ), or pubertal status ( $t[41]=-0.445$ ,  $p=.659$ ). As expected, the group with BD had higher YMRS ( $t[41]=-6.95$ ,  $p<.001$ ) and CDRS scores ( $t[41]=-7.36$ ,  $p<.001$ ) and lower CGAS global functioning ratings ( $t=12.98$ ,  $p<.001$ ) than did the HC group. Measures of anxiety in the MASC physical domain were significantly higher in the group with BD ( $t=-2.89$ ,  $p=.007$ ) than in the HC group, but there were no significant group differences in the domains of harm avoidance, social anxiety, or separation anxiety. The group with BD had significantly higher scores on trait impulsivity than did the HC group in the attention ( $t=-2.95$ ,  $p=.006$ ), motor ( $t=-3.50$ ,  $p=.001$ ), and non-planning ( $t=-4.19$ ,  $p>.001$ ) domains. Finally, as reported previously,<sup>38</sup> there were no significant between-group differences in the endorsement of or memory for positive and negative adjectives.

### Group Differences in Seed-Based Amygdala-Subregion Networks

Compared to HC youth, youth with BD had significantly less positive connectivity between the right laterobasal amygdala and the right hippocampus. Youth with BD also had greater anticorrelation than did HC youth between the right laterobasal amygdala and the right precentral gyrus. Finally, youth with BD also had significantly more positive connectivity than did HC youth between the right laterobasal amygdala and the left precuneus (see Table 2 and Figure 1). These results held even after correcting for multiple comparisons. No other



group differences were obtained by seeding left laterobasal, right or left centromedial, or right or left superficial amygdala subregions.

### **Exploratory Associations Between Amygdala Subregion Connectivity, Clinical Symptom Domains, and Memory**

To ground the obtained group differences in amygdala subregion connectivity in terms of clinical assessment, we explored the relations between these differences in connectivity and key clinical symptom domains and emotional memory that are subserved by neural regions that are connected to the amygdala subregion. We found no significant correlations between connectivity estimates and age, mania, depression, or trait impulsivity within each group. Across both groups, greater recall of negative words was correlated with reduced connectivity between right laterobasal amygdala and right hippocampus ( $r[38]=-0.343$ ,  $p=.035$ ); the two groups did not demonstrate significantly different relations between connectivity estimates and estimates of emotional memory. In youth with BD, however, higher levels of separation anxiety were related to stronger connectivity between the right laterobasal amygdala and right hippocampus ( $r[17]=0.627$ ,  $p=.007$ ); this was not the case in HC youth ( $r[21]=-0.21$ ; Fisher's  $R$  to  $Z = 2.66$ ,  $p=.008$ ). We also found a strong correlation of poorer insight and increased connectivity between right laterobasal and left precuneus within the group with BD ( $r[18]=0.482$ ,  $p=.04$ ). As expected, there was little variability in insight within the HC group. All of these correlations remained statistically significant even after controlling for mania and depression symptom dimensions. No other correlations were found between other symptom or cognitive variables and connectivity in the right laterobasal amygdala.

### **Exploratory Medication Analyses**

Within the group with BD, amygdala subregion connectivity estimates did not differ in participants taking lithium ( $n=8$ ) vs. participants not taking lithium ( $n=12$ ), in participants taking antipsychotics ( $n=12$ ) vs. participants not taking antipsychotics ( $n=8$ ), or in participants taking mood stabilizers ( $n=8$ ) vs. participants not taking mood stabilizers ( $n=12$ ). Because of the small number of participants who had taken specific medications, we could not reliably assess medication effects separately for participants taking antidepressants ( $n=4$ ), stimulants ( $n=2$ ), or anxiolytics ( $n=1$ ). It is noteworthy, however, that removing these youth from the main analyses did not change the findings reported in this paper.

## **DISCUSSION**

This study was designed to examine differences in amygdala subregion resting state functional connectivity between youth with BP-I and typically developing controls. Although youth with BP-I did not demonstrate predicted aberrant connectivity between the amygdala and the VLPFC, ACC, or insula, they did exhibit anomalous patterns of resting state functional connectivity between the laterobasal amygdala subregion and other regions critical for the processing and regulation of emotion. Consistent with a recent shape analysis of the amygdala that localized volumetric abnormalities in pediatric BD to the laterobasal subdivision,<sup>45</sup> neither centromedial nor superficial subdivisions differentiated youth with BD from HCs. Youth with BD exhibited lower amygdala laterobasal to prefrontal and

hippocampal connectivity, but greater amygdala laterobasal to precuneus connectivity than did HC youth. Correlational analyses suggest that this profile of aberrant amygdala connectivity observed at rest in youth with BD represents mood-related disruptions in memory, anxiety, and insight in these participants.

The regional connectivity in the right laterobasal amygdala that we documented in this study is consistent with patterns previously described in healthy adults.<sup>23</sup> Specifically, healthy adults show greater functional connectivity between the laterobasal amygdala and temporal regions, including the hippocampus, than they do for either the centromedial or the superficial subdivisions of the amygdala. Connectivity with temporal regions, together with increased connectivity to prefrontal regions, including the precentral gyrus, has been implicated in facilitating associative learning and emotional memory.<sup>46–48</sup> In the present study, youth with BD, compared with healthy controls, showed decreased connectivity between the laterobasal subdivision and both the hippocampus and the precentral gyrus, suggesting a vulnerability for functional impairments in learning and memory subserved by these regions. Indeed, youth with BD have well-documented impairments in multiple domains of memory function,<sup>49,50</sup> reduced hippocampal volumes,<sup>51</sup> and dysfunction in brain networks that support memory function.<sup>52,53</sup> Task-based functional abnormalities in the precentral gyrus have also been reported in adults with BD during autobiographical recall.<sup>54</sup> We have previously demonstrated that youth with BD endorse and recall more negative self-referent adjectives than do healthy youth, and that depressive symptomatology is associated with impaired memory for positive self-referent adjectives.<sup>38</sup> In the present study, greater recall of negative self-referent words was associated with weaker laterobasal-hippocampal connectivity, although HC participants and those with BD did not differ in this relation. Further studies with larger samples are needed to confirm whether negatively valenced emotional material interferes with encoding and recall of stimuli in youth with BD.<sup>55</sup> Importantly, those youth with BD in the present study who had higher levels of separation anxiety also had stronger laterobasal-hippocampal connectivity, an association consistent with a neural model of aversive learning or threat conditioning that predisposes adolescents to develop anxiety disorders.<sup>56</sup> In this context, it is noteworthy that anxiety is the most common comorbidity in pediatric BD<sup>57</sup> and adversely affects the course of BD among youth.<sup>58</sup>

Interestingly, youth with BD showed greater resting-state connectivity than did HC youth between the laterobasal amygdala nucleus and the precuneus that has been found in healthy adults to show more negative than positive functional connectivity with the amygdala.<sup>23,59</sup> In healthy controls, this opposing pattern of connectivity between the precuneus and the amygdala serves to mediate cognitive and affective processes critical for emotional regulation. In contrast, a preponderance of fMRI studies using emotional stimuli with individuals with BD has found hyperactivity in both the amygdala and precuneus, particularly in youth with BD.<sup>14</sup> Hyperactivity of the amygdala and precuneus in response to emotion processing in BD may be explained by the relative intrinsic hyperconnectivity of these two structures at rest.<sup>60,61</sup> The present findings are consistent with results of a recent study that found greater connectivity between the laterobasal amygdala and precuneus in youth with “narrow phenotype” BD than in youth with severe mood dysregulation and healthy controls.<sup>62</sup> In the present study we found further that laterobasal amygdala and

precuneus hyperconnectivity in youth with BD was correlated with poorer insight about their illness, supporting an interpretation that hyperconnectivity between these regions is maladaptive. Indeed, poor insight about their illness has consistently been described as a disruption in self-awareness in patients both with schizophrenia<sup>63</sup> and with BD;<sup>64</sup> we know little, however, about the neurobiological basis of this phenomenon.

The abnormalities in resting state connectivity involving the amygdala that we documented here in youth with BD overlap with the profile of resting state functional connectivity found in adults and adolescents with BD<sup>17,18,65–67</sup> and in amygdala default mode network connectivity correlates with depression severity in dysthymic adults<sup>68</sup>; together, these findings support models of limbic dysfunction across mood states in BD. Decreased amygdala-hippocampal and increased amygdala-precuneus connectivity was also recently reported in adolescents with unipolar depression compared to healthy controls,<sup>69</sup> suggesting that these connectivity patterns do not represent biomarkers that are specific to unipolar or bipolar forms of depression among youth. Rather, this overlap likely represents a common pattern of amygdala dysfunction in youth with mood disorders.<sup>70,71</sup> One recent resting state study in adults with BD found that patterns of amygdala-hippocampal connectivity differentiated manic from depressed mood states; whereas increased connectivity was observed in bipolar mania, decreased connectivity was found in bipolar depression.<sup>72</sup> Additional studies with larger sample sizes of subgroups with manic and depressed bipolar disorder are needed to determine whether this pattern also characterizes youth, and whether this finding has predictive value or significant implications for treatment.<sup>73</sup>

We should note three limitations of this study. First, this study was cross-sectional and included a relatively small, heterogeneous sample of medicated youth with BD. Importantly, however, covarying for symptoms of mania and depression and comparing subgroups of youth with and without exposure to major classes of medication did not alter our findings. Exploratory subanalyses of various medication exposures were limited by sample size and did not control for duration of treatment. Nevertheless, in this context, the current body of relevant literature suggests that medication exposure in BD is more likely to have a normalizing effect on neural function than it is to cause new abnormalities that might confound MRI results.<sup>74</sup> Second, in this study, we focused solely on youth in late adolescence with BD; the addition of a group of youth diagnosed with a different disorder, such as major depressive disorder, and across developmental stages (younger youth and adults) would help to elucidate the specificity of the obtained findings to BD and to specific stages of development. Third, our use of a relatively low cluster-forming threshold ( $Z=2.3$ ) may have resulted in missing significant activation within the context of a large ROI.<sup>75</sup> It is important to note, however, that our results did not yield significantly large clusters, nor did they have ambiguous anatomical boundaries. Further, we used thresholds comparable to those reported in previously published analyses examining amygdala subregion connectivity.<sup>23</sup> Despite these limitations, however, we did document differences between HC youth and those with BD in the intrinsic connectivity of the laterobasal amygdala subregion with key brain regions that are associated with learning, memory, and self-awareness, as well as differences in the relation of this connectivity with separation anxiety that is common among youth with BD.

In this study, we present evidence that early in the course of their illness, youth with BD exhibit anomalies in functional connectivity in the laterobasal subdivision of the amygdala at rest. Specifically, we found a prominent role of aberrant functional connectivity with the precentral gyrus, hippocampus, and precuneus, correlating with relevant functional and clinical domains of socioemotional learning, anxiety, and self-referential insight. Future research is needed to examine the longitudinal trajectories of these characteristics and their ability to predict outcomes in youth in the early stages of BD.

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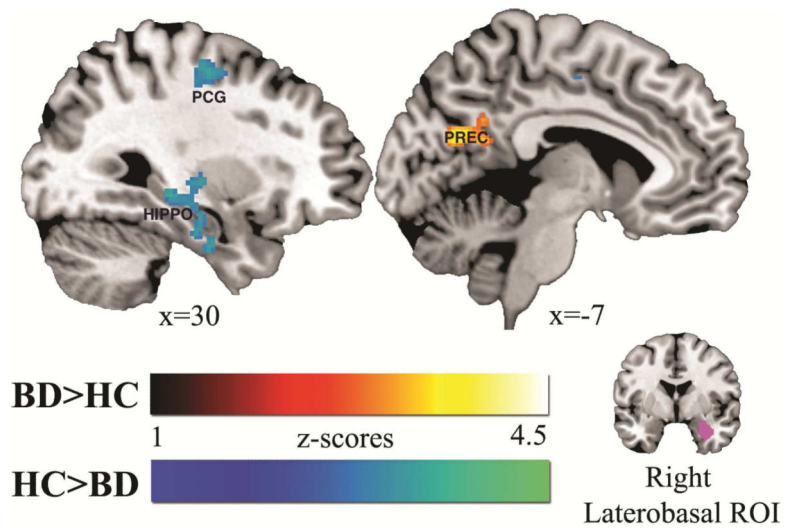
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### Clinical Guidance

- Youth with bipolar disorder (BD) show aberrant patterns of intrinsic laterobasal amygdala connectivity at rest.
- Compared with typically developing healthy controls, youth with BD have decreased connectivity between the laterobasal subdivision of the amygdala and the hippocampus and precentral gyrus, and increased connectivity between the laterobasal subdivision and the precuneus.
- Connectivity between the right laterobasal amygdala and right hippocampus is positively correlated with levels of anxiety in youth with BD but not in HC youth, and connectivity between the right laterobasal amygdala and right precuneus is negatively correlated with insight about bipolar illness.
- Together, disruptions in amygdala connectivity may manifest clinically in youth-onset BD as abnormal modulation of mood and may distinguish psychopathology from processes associated with typical adolescence.



**Figure 1.** Functional connectivity of the right laterobasal amygdala region of interest (ROI). Note: Patterns of significant relations in youth with bipolar disorder relative to healthy controls (BD>HC) in the left precuneus (PREC) are shown in warm colors, and in healthy controls relative to youth with bipolar disorder (HC>BD) in the right precentral gyrus (PCG) and right hippocampus (HIPPO), in cool colors.

**Table 1**

## Demographic and Clinical Variables of Study Sample

Variable	Bipolar n=20		Healthy Control n=23	
	Mean (SD)	SD	Mean (SD)	SD
Age, years, mean (SD)	17.21	1.89	16.86	1.43
Gender, Female, n (%)	11	55%	14	60.8%
Right Handedness, n (%)	18	90	21	91
Caucasian Race, n (%)	11	55	13	56.5
Full Scale IQ, mean (SD)	108.00	7.79	111.52	9.97
YMRS, mean (SD) *	14.15	9.16	0.78	1.17
CDRS, mean (SD) *	40.15	13.54	19.17	1.85
CGAS, mean (SD) *	90.76	4.53	60.35	9.49
MASC physical, mean (SD)	8.50	6.85	3.57	3.34
MASC Harm Avoidance, mean (SD)	13.25	4.56	15.14	3.05
MASC Social Anxiety, mean (SD)	10.56	6.25	7.76	4.90
MASC Separation Anxiety, mean (SD)	2.68	3.52	2.76	2.30
BIS Attention, mean (SD)	17.50	3.65	13.90	3.85
BIS Motor, mean (SD)	24.17	5.33	19.40	2.81
BIS Non-planning, mean (SD)	29.00	5.54	22.35	4.21
Current Medication, n (%)				
Lithium	8	40%	0	0
Antipsychotics	12	60%	0	0
Antidepressants	4	20%	0	0
Anticonvulsants	8	40%	0	0
Stimulants	2	10%	0	0
Anxiolytics	1	5%	0	0

Note: BIS = Barratt Impulsiveness Scale; CDRS = Childhood Depression Rating Scale; CGAS = Clinical Global Assessment Scale; MASC = Multidimensional Anxiety Scale for Children; YMRS = Young Mania Rating Scale.

\* p<.05

**Table 2**

Between-Group Differences Seeding the Right Laterobasal Amygdala Subregion

Group difference	Cluster Size (voxels)	Region	Z-scores	MNI coordinates			p-value (FWE)
				x	y	z	
HC>BD	595	Right precentral gyrus	3.36	26	-14	54	p<.001
HC>BD	454	Right hippocampus	3.86	38	-28	-10	p<.005
BD>HC	407	Left precuneus	3.53	-6	-64	24	p<.010

Note: BD = Youth with bipolar-I disorder; FWE = familywise error rate; HC = healthy control youth; MNI = Montreal Neurological Institute.