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# Reduced Functional Connectivity of Prefrontal Regions and Amygdala Within Affect and Working Memory Networks in Pediatric Bipolar Disorder

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## Abstract

This study examined whether adolescents with pediatric bipolar disorder (PBD) have abnormal regional functional connectivity in distributed brain networks during an affective working memory task. Adolescents with PBD ($n = 41$) and healthy controls (HC; $n = 16$) performed a two-back functional magnetic resonance imaging working memory task with blocks of either angry or neutral faces. Independent component analysis methodology identified two temporally independent and functionally connected brain networks that showed differential functional connectivity in PBD and HC. Within a network for "affect evaluation and regulation," PBD showed decreased functional connectivity relative to HC in regions involved in emotion processing such as the right amygdala, and in emotion regulation regions such as the right ventrolateral prefrontal cortex (VLPFC), while functional connectivity was increased in emotion evaluation regions such as the bilateral medial PFC. Furthermore, in an "Affective Working Memory Network," PBD exhibited greater connectivity relative to HC in left dorsolateral PFC (DLPFC), caudate, and right VLPFC; and simultaneously reduced connectivity in emotion processing regions, such as the right amygdala, bilateral temporal regions, and the junction of DLPFC/VLPFC, which interfaces affective and cognitive processes. Dysfunction in network engagement in PBD patients illustrates that they are expending greater effort in face emotion evaluation, while being less able to engage affect regulation regions.

**Key words:** affect; bipolar; functional connectivity; independent component analysis; pediatric; working memory

## Introduction

Pediatric bipolar disorder (PBD) is a pediatric illness with persistent affect dysregulation that affects about 2% of children (Van Meter et al., 2011). The narrow phenotype of PBD, Type I and II (DSM IV-TR) (American Psychiatric Association, 2000), presents with mania and hypomania, elation, grandiosity, irritability, racing thoughts, decreased need for sleep, and hyper-sexuality (Geller et al., 1998; Leibenluft et al., 2003). In these children, the persistent affect dysregulation is often accompanied by severe cognitive impairment (Passarotti and Pavuluri, 2011; Pavuluri et al., 2006), leading to considerable deficits in social interactions, self-regulation, and cognitive and school functioning (Pavuluri et al., 2006; 2009b). In particular, working memory, the ability to temporarily store and manipulate information in short-term memory, is often severely impaired in PBD (Dickstein et al., 2004; Frazier et al., 2005; Pavuluri et al., 2006), with deficits persisting over development and leading to lower academic achievement relative to age-matched peers, even in euthymic patients (Pavuluri et al., 2009a, 2010).

In this study, we wished to characterize the brain regions that form functionally integrated networks which support the interface of working memory and affect processing, and to test for connectivity abnormalities in adolescents with PBD relative to healthy controls (HC). Working memory functions strongly rely on prefrontal brain regions, such as the dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC), dorsal anterior cingulate cortex (ACC), as well as basal ganglia and posterior temporoparietal regions (D'Esposito, 2007; Owen et al., 2005). There is also some evidence that the junction of DLPFC and

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VLPFC is involved in processes at the interface of cognition and affect (Petrides and Pandya, 2002). Several recent functional magnetic resonance imaging (fMRI) studies have indicated that the aforementioned regions are also implicated in PBD pathophysiology (Passarotti and Pavuluri, 2011; Passarotti et al., 2010a, 2010b, 2010c, 2011; Pavuluri et al., 2010).

To date, researchers are still investigating the mechanisms by which working memory is influenced by emotions (Dolcos and McCarthy, 2006). This is a particularly important question when studying PBD dysfunction, given that this pediatric illness presents with a complex interaction of affective and cognitive deficits. In fact, emotional stimuli engender interference in performance and affect working memory circuits in healthy individuals (Dolcos and McCarthy, 2006). However, the effects of emotional challenge on the neural bases of cognition are worse in the PBD population compared with healthy peers (Passarotti et al., 2010a, 2010c, 2011; Pavuluri et al., 2008; Rich et al., 2008). For instance, previous fMRI studies with smaller samples of acutely ill PBD patients found reduced cortical activity in VLPFC (Passarotti et al., 2010c, 2011) and increased amygdala activation (Passarotti et al., 2011) relative to HC during an affective working memory task with angry faces, suggesting dysfunction at the interface of working memory and affect circuits that is worsened in the presence of negative emotions in PBD. Nevertheless, the biological mechanisms of the underlying dysfunction in PBD are not well-understood (Passarotti et al., 2010c, 2011; Pavuluri et al., 2008; 2010).

While traditional fMRI time-series analyses in block design or event-related studies have been very useful in identifying brain functional differences between PBD and HC in isolated cortical and subcortical brain regions, we decided to further investigate the putative dysfunction at the interface of affect and cognition in PBD in terms of network functional connectivity. Functional connectivity methods (Friston, 2002; McIntosh et al., 1993) can provide a further understanding of how brain regions were found to be over- or under-activated in PBD, while traditional fMRI analyses may be over- or under-engaged in large-scale, distributed neural networks involved in affective and cognitive processing. Initial evidence from adult BD studies using psychophysiological interaction analyses suggests reduced VLPFC regulation of amygdala response during an emotional labeling task (Foland et al., 2008). Moreover, resting-state seed voxel analysis methods found greater negative correlation in the VLPFC-amygdala connectivity in HC relative to BD (Chepenik et al., 2010) and, in addition, decreased resting-state connectivity between pregenual ACC and amygdala (Anand et al., 2009). These studies suggest decreased connectivity in amygdala and cortico-limbic circuits involved in mood regulation in BD relative to HC. Furthermore, using dynamic causal modeling, Almeida and associates (2009) found evidence of dysfunction in ventromedial systems involved in stimulus evaluation in BD. To date, there are only two published studies on functional connectivity in pediatric population with BD relative to HC, and they suggest altered functional connectivity between limbic and temporal regions during face emotion identification (Rich et al., 2008) and between the frontal and temporal circuit during the resting state (Dickstein et al., 2010). However, so far, no study has investigated functional connectivity in PBD in a task at the interface of cognitive and affective systems. To our knowledge, this is the first whole-brain functional connectivity study conducted on functional networks underlying the interaction between affective and working memory systems in PBD.

Based on the initial indications of altered functional connectivity in PBD, we used a well-established functional connectivity method, independent component analysis (ICA) (Calhoun et al., 2001; Stevens et al., 2007, 2009), to study the differences between PBD and HC in large-scale distributed neural network connectivity. The advantages of ICA methodology include that unlike typical fMRI analyses, ICA identifies spatially independent components within blood oxygenation level-dependent (BOLD) time series data in a data-driven manner, without the need for a task reference function to separate signal from noise (Calhoun et al., 2001; Stevens et al., 2007, 2009). Participants performed a two-back affective working memory task, which typically engages fronto-striato-parietal networks (Owen et al., 2005), where stimuli were angry and neutral faces. For the purpose of this study, we focused on negative-valence stimuli such as angry faces, rather than positive-valence stimuli, because negative-valence stimuli have been more effective in identifying neural markers of manic state in PBD (Passarotti et al., 2010a, 2010c, 2011; Pavuluri et al., 2008).

We hypothesized that the data-driven ICA analyses would reveal functionally segregated networks related to face processing, working memory, and affect processing and regulation, encompassing regions found with previous conventional fMRI studies using similar paradigms, such as an emotion processing network (i.e., amygdala), an emotion evaluation network (including medial PFC and insula), an emotion regulation network (including VLPFC and ventral ACC) (Foland et al., 2008; Passarotti et al., 2010a, 2010b; Pavuluri et al., 2008), a Working Memory Network, including fronto-striatal circuits (D'Esposito, 2007; Owen et al., 2005; Passarotti et al., 2010a, 2010c), and a face processing network (Haxby et al., 2002; Passarotti et al., 2007). We hypothesized that PBD patients relative to HC would show differences in regional functional connectivity in these brain networks, especially in regions involved in emotion processing, emotion evaluation, and emotion regulation, as described earlier. Moreover, we expected that in PBD dysfunctional connectivity in affective and regulatory regions may correlate with the severity of manic and depressive symptoms and/or with performance measures on the affective working memory task.

## Methods

### *Participants*

Our adolescent sample (mean age = 14.32 ± 2.76) consisted of 41 un-medicated adolescents with PBD (type I: $n = 34$; Type II: $n = 7$) and 16 HC. For this sample, the age range was from 11 to 18 years. We made every effort to match the PBD and HC groups for IQ, as estimated with the Wechsler Abbreviated Scale of Intelligence (WASI) (Psychological Corporation, 1999), age, socioeconomic status (SES), gender, race, and handedness as measured by a handedness questionnaire (Annett, 1970).

Our participants were recruited from the child psychiatry clinics at the University of Illinois at Chicago (UIC) and from community referrals. HC were recruited from the neighboring community through an advertisement. This study was approved by the UIC Institutional Review Board and was

undertaken with the understanding and written consent of each participant. We obtained an assent for children younger than age 15, and an informed consent for adolescents aged 15 or older. Consent from at least one parent or legal guardian was always obtained.

Diagnostic assessment. PBD diagnosis was obtained based on DSM-IV diagnosis of bipolar disorder with mixed, manic, or hypomanic episode (DSM-IV, 2000). Moreover, the child participant and a parent or legal guardian were interviewed by a board-certified child psychiatrist (M.N.P.) and two board-certified doctoral-level clinicians within our research program, using the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al., 1998), supplemented by the episode characterization of bipolar disorder from the KSADS - Present and Lifetime version (Kaufman et al., 2000). In addition, the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Child Depression Rating Scale-Revised (CDRS-R) (Poznanski et al., 1984) assessed manic and depressive symptom severity, respectively. All available clinical information was reviewed to make a consensus clinical diagnosis. Live diagnostic interviews of ten cases were independently coded by two researchers to establish inter-rater diagnostic reliability (Cohen's kappa = 0.94).

Inclusion and exclusion criteria. Inclusion criteria for participants with PBD were as follows: 10–18 years of age, a baseline score greater than 12 on the YMRS (Young et al., 1978), and consent to be scanned in a medication-free state. Patients were either medication free (not requiring a washout at study entry) or sufficiently unstable on previous medications to justify discontinuation of an ineffective treatment before beginning a new treatment with the consent of parents and the assent of patients. The washout period consisted of tapering previous medications over 1 week before study entry, except for those who received aripiprazole that required a 4-week washout period. All patients were medication free for at least 7 days before scanning. None of the patients were on fluoxetine that would have required a longer washout period. Close clinical supervision and monitoring was provided during drug free periods according to the approved IRB protocol. Inclusion criteria for the HC participants included being 10–18 years of age and having a baseline YMRS score $< 12$. Exclusion criteria for all participants included: current substance abuse/dependence, neurological complications, serious medical illness, full-scale IQ $< 70$, and contraindications to MRI studies, including metallic implants, braces or retainers, and claustrophobia. The HC participants were excluded if they met criteria for a DSM-IV Axis I disorder or had a family history of affective illness.

Behavioral and ICA data from the present samples of 16 HC and 41 PBD patients have not been published earlier.

### *The affective two-back working memory task and fMRI session*

Each participant underwent a 7 min fMRI scanning session with a two-back working memory task. While the scanning session included two tasks, one with blocks of angry and neutral faces and another with blocks of happy and neutral faces, for the purposes of this study we will focus only on the angry and neutral face task blocks. Our face stimuli were 80 Gur emotional faces (Gur et al., 2002) with neutral and angry expressions that were balanced by gender, race, and facial expression.

This task consisted of four alternating 30-sec blocks of angry and neutral faces presented in a pseudo-random sequence. Each block consisted of ten trials. On each trial, a face stimulus with a certain emotion (i.e., angry or neutral) was presented for 3 sec and then disappeared, followed by the next face for 3 sec. Participants responded by key press if they saw the same emotional face (i.e., same face and emotion), as the one presented two trials earlier (Fig. 1). In this task, a two-back match trial always involved a match in both face identity and face emotion. A 20 sec fixation in between blocks was included to allow for emotional arousal to return to baseline. A color high-resolution LCD projector projected visual stimuli onto a rear projection screen that was viewed via an angled double-mirror system mounted on a standard General Electric head coil. A camera monitored each participant's right eye during the scan to ensure that participants were looking at the visual stimuli.

### *MRI protocols*

Gradient-echo, echo-planar functional imaging, and structural acquisitions were performed with a 3.0 Tesla whole-body scanner (Signa; General Electric Medical System, Milwaukee, WI) at the MR Center within the UIC Hospital. To minimize head motion, we restricted the participants' head with foam cushions. T2*-weighted functional images were acquired with a gradient-echo, echo-planar sequence (TR = 2500 ms, TE = 25 ms, flip angle = 90°, FOV $20 \times 20\,cm^2$, $64 \times 64$ matrix, $3.125 \times 3.125$ mm in plane resolution, 4-mm slice thickness, 1-mm gap, 25 slices). Anatomical images were also acquired in the axial plane (three-dimensional [3D] spoiled gradient recalled, 1.5 mm-thick contiguous axial slices, in plane resolution = $0.47 \times 0.47$) and were later coregistered with the functional data. The experiment run consisted of 168 time points, including a 5 sec rest session at the beginning that was collected to allow for $T_1$ effects to stabilize.

### *fMRI image processing and motion correction*

FIASCO software (Functional Imaging Analysis Software—Computational Olio) (Eddy et al., 1996) was used to implement 3D motion estimation and correction, removal of slow signal drift, and identification of images with artifacts such as high shot noise or displacement that cannot be readily corrected by motion correction algorithms. We excluded from the analyses individual volumes from the time series if head displacement from the median head position was greater than 1.5 mm, or if head rotation from the median head position was greater than 0.5°. Motion correction and de-trending were performed using FIASCO. Based on analyses done on FIASCO output, the two groups did not differ significantly ($p > 0.05$) for mean motion during the task. In addition, there were no significant group differences ($p > 0.05$) in the number of volumes retained after discarding those with motion artifact.

After motion correction and de-trending using FIASCO, the functional images were preprocessed with SPM5 (www.fil.ion.ucl.ac.uk/spm/software/spm5/). Slice timing correction was applied to the data in order to remove signal

variation due to differences in slice acquisition temporal onset, and to ensure that the data from each slice corresponded to the same time point. The first functional image volume of each participant was used to determine the parameters for spatial normalization into Montreal Neurological Institute (MNI) standardized space employed in SPM5 using nonlinear transformation. The normalization parameters determined for the first functional volume were subsequently applied to all of the 168 functional image volumes for each participant.

### *ICA estimation*

All participants' fMRI time series for the affective N-back task were analyzed using a group ICA algorithm (GIFT v1.3h; http://icatb.sourceforge.net) (Calhoun et al., 2001). The fMRI time series data for all participants were concatenated and then subjected to two principal component analysis data reduction stages (Calhoun et al., 2001). The data underwent a final ICA rotation using Infomax that produced 46 maximally independent components (Bell and Sejnowski, 1995). The minimum description length criterion was used to determine the number of components (Li et al., 2007). Using the ICA-derived group solution, data for each participant were then back-reconstructed (Erhardt et al., 2010) so that individual participant variability was retained for hypothesis testing. For each component, this back-reconstruction method produced a spatial map representing brain regions within each component "network," and a time course of BOLD signal change across the fMRI paradigm.

### *Selection of components for analysis*

ICASSO analyses (Li et al., 2007) in GIFT were run 30 times using FastICA in order to investigate signal coherence and replicability for each of the estimated 46 independent components, and to identify those with acceptable reliability (i.e., >80%) that could be retained for further analysis. Next, the correlation of each component's spatial map with *a priori* probabilistic maps of gray matter, white matter, and cerebral spinal fluid (CSF) within MNI space (templates provided in SPM5) was calculated for all components in order to discard those that could be an artifact, because they had greater than a 0.25 correlation to CSF or white matter, or which showed a low correlation with gray matter. This step primarily identified and excluded obvious signal artifacts (e.g., head motion, cardiac inflow pulsatile motion). Of the components that were retained through this process, we proceeded by discarding components in which there was no evidence that they were engaged by any aspect of the fMRI task. To assess task engagement, multiple regression analyses were performed between component time courses and an overall condition model of the affective N-back task (i.e., one condition model for both angry and neutral blocks) to provide association coefficients ($\beta$-weights). One sample *t*-test against zero was carried out on the $\beta$ weights (pooled across groups) to determine whether the evidence for task engagement was greater than zero. By doing so, 26 components were significantly ($p<0.05$) associated with the overall-condition model and were, therefore, retained. Finally, to select a final subset of components on which to test our hypotheses, we compared $\beta$-weights representing the degree of task engagement between HC and PBD groups. Twenty-six ANOVAs were performed, one for each of the retained components. The mixed-factor ANOVA model included group (PBD, HC) as the between-subjects factor, and face emotion (angry, neutral) as the within-subject factor. Components for which there was evidence for significant ($p<0.05$) group, valence or group × valence interactions were retained for study hypothesis testing so that we could test differences between PBD and HC in regional functional connectivity with regard to networks involved in the integration of working memory and face emotion processing.

### *Visualization of whole-brain, task-engaged components*

For each component that exhibited significant effects from the ANOVA, component spatial structure was identified using an SPM5 voxel-wise, one-sample *t*-test across all study participants ($p<0.01$ family-wise error rate) and visualized by overlaying these results on axial slices of representative brain anatomy.

### *Study hypothesis testing*

Primary analyses. To test our hypotheses about group differences in functional connectivity, two-sample *t*-tests in SPM5 were performed on the spatial maps depicting network structure in each participant. These analyses determined whether the PBD group had any regional deficits or excesses of functional connectivity relative to the HC group. In order to correct for multiple voxel-wise comparisons, we adopted AlphaSim cluster thresholding (Ward, 2000), restricted to in-brain voxels, which used a contiguity threshold (minimum cluster size = 11; uncorrected $p=0.01$) that ensured an experiment-wise, Type 1 error rate of $p<0.02$ (corrected $p$). Next, we identified clusters of voxels with a minimum size of 11 voxels that exhibited significant group differences at a corrected $p<0.02$ in the *t*-test maps.

Secondary analyses. We also conducted a series of secondary analyses to better characterize (i) the relevance of within-group regional connectivity differences based on task conditions (i.e., angry and neutral faces), and (ii) correlations between voxel-wise regional connectivity values and clinical or behavioral measures. First, a supplemental SPM5 correlation analysis examined the linear association of individual component spatial maps to each participant's $\beta$-weights (representing the degree of component engagement) for angry or neutral faces. This analysis showed which brain regions of any given component were specifically implicated in the "fit" of its time course to the canonical hemodynamic response model for angry or neutral blocks in each group separately. In this way, it was possible to see whether or not a particular region's functional connectivity was more or less important to processing angry or neutral valence in faces (i.e., more strongly implicated in PBD network connectivity abnormality).

Next, SPM5 correlation analyses examined relationships between clinical measures (i.e., YMRS, CDRS scores) and spatial maps for individual PBD participants for each of the considered networks. Finally, we performed SPM5 correlation analyses between network maps in each group and average median response time (RT) and accuracy to determine whether brain connectivity within each network overtly influenced neuropsychological function. Bonferroni

corrections and AlphaSim cluster thresholding (Ward, 2000) were applied to correct for multiple comparisons (See details in Methods section).

### *Working memory task-performance analyses*

A repeated-measures ANOVA with group (PBD, HC) as a between-subjects factor, and face emotion (angry, neutral) as a within-subjects factor, was carried out on median RT and accuracy data. Median RT was used instead of mean RT, because the former is much less influenced by outliers and by high RT variability that is often present in a pediatric psychiatric population.

## Results

### *Demographic and clinical data*

Table 1 shows demographic and clinical data for the PBD and HC groups. Separate ANOVAs for each of the demographic measures revealed no significant group differences for age, estimated IQ and SES. Using two-tailed Fisher's p tests, we also found no significant group differences for handedness, gender, and sample racial composition. With regard to the clinical scales, as expected, relative to HC the PBD group had higher YMRS [$F(1,55) = 73.13$, $p < 0.0001$] and CDRS-R [$F(1,55) = 94.31$, $p < 0.0001$] scores.

### *Behavioral performance results*

Median RT and accuracy for the task in each group and condition are presented in Table 2. For median RT, there was a significant main effect of group [$F(1,55) = 13.49$, $p < 0.0005$] in that overall median RT in PBD (982 ms) was significantly slower than in HC (793 ms). With regard to accuracy, there was only a main effect of group [$F(1,55) = 5.84$ $p < 0.02$] in that PBD (96%) had lower accuracy than HC (99%). No other significant results were found.

### *Primary analyses: component networks.*

The temporal regression analyses found three functional whole-brain networks that had either a group, or valence or group × valence interaction, indicating relevance to our study objectives (Table 3). In the spatial maps for each of these functional networks (represented in Figs. 2–4), the increase (represented with the lighter gray, or color red in the figures) or decrease (represented with the darker gray, or color blue in the figures) in engagement within the network represents the directionality of functional connectivity (i.e., positive or negative) across all participants (i.e., patients and HC) during performance of the n-back working memory task. Since we had uneven samples, to ensure that there were no significant group differences in variance that may affect

Table 1. Demographic and Clinical Characteristics of Patients with Pediatric Bipolar Disorder and Healthy Control

| | *PBD* (n = *41*) | *HC* (n = *16*) | *Analyses* |
|---|---|---|---|
| *Variables* | *Mean (SD)* | *Mean (SD)* | *(F), p-value* |
| Age (years) | 14.00 (2.31) | 14.63 (3.2) | *(0.67), p = 0.42* |
| WASI- *FSIQ*[a] | *102.00 (8.15)* | *106.00 (10.36)* | *(3.51), p = 0.07* |
| Socioeconomic Status[b] | 2.34 (.62) | 2.06 (.85) | *(1.88), p = 0.17* |
| YMRS | 19.89 (8.62) | 1.25 (1.51) | *(73.13), p = 0.00001* |
| CDRS | 51.97 (13.19) | 19.58 (2.29) | *(94.31), p = 0.00001* |
| | n *(%)* | n *(%)* | *Fisher exact* p-*value (two-tailed)* |
| Sex | | | *p = 0.77* |
| Male | 16 (39%) | 7(44%) | |
| Female | 25 (61%) | 9(56%) | |
| Race | | | *p = 0.24* |
| Caucasian | *20 (49%)* | *11 (69%)* | |
| *Other* | *21 (51%)* | *5 (31%)* | |
| *Handedness* | | | *p = 0.31* |
| Right-handed | 36 (88%) | 16 (100%) | |
| Left-handed | 5 (12%) | 0 (0%) | |
| Episode | | | |
| Manic | 26 | - | |
| Mixed | 10 | - | |
| Hypomanic | 5 | - | |
| Comorbidity | | | |
| ADHD | 14 | - | |
| Psychosis | 3 | - | |
| GAD | 5 | - | |
| ODD | 4 | - | |

[a]Wechsler Abbreviated Scale of Intelligence Intelligent Quotient (WASI IQ; Matrix Reasoning and Vocabulary Subtests).
[b]Mean revised Hollingshead socioeconomic status.
PBD, pediatric bipolar disorder; HC, healthy control; YMRS, Young Mania Rating Scale; CDRS-R, Child Depression Rating Scale-Revised; ADHD, attention-deficit hyperactivity disorder; GAD, generalized anxiety disorder; ODD, oppositional-defiant disorder.

Table 2. Median Response Time and Accuracy for the Two-Back Working Memory Task in Patients with Pediatric Bipolar Disorder and in Healthy Controls

| | *PBD* (n = *41*) | *HC* (n = *16*) |
|---|---|---|
| Median RT (in ms) | Median (SD) | Median (SD) |
| Angry face emotion | 948 (163) | 817 (130) |
| Neutral face emotion | 1016 (268) | 768 (161) |
| Total average[a] | 982 (216) | 793 (146) |
| Accuracy (% correct) | % (SD) | % (SD) |
| Angry face emotion | 96 (3) | 99 (2) |
| Neutral face emotion | 96 (3) | 98 (2) |
| Total average[b] | 96 (3) | 99 (2) |

[a]Significant group effect ($p = 0.0005$) for RT.
[b]Significant group effect ($p = 0.02$) for accuracy.
RT, response time.

the results, we carried out Levene's tests for equality of variances (which assesses the equality of variances in different samples using the mean of the sample) for our components, and we found no significant group differences in variance ($p = 0.32$) in any component.

Face Emotion Processing Network. We identified a component that showed a significant effect of face emotion valence [$F(1,55) = 4.85$, $p = 0.03$] but no significant effects of group ($p > 0.05$) or interaction of group × face emotion valence ($p > 0.05$) (Table 3). This spatial network comprised regions involved in perceptual processing of facial features and emotion (Haxby et al., 2002; Passarotti et al., 2007; Pavuluri et al., 2008), including occipital regions, fusiform gyrus, parahippocampal gyrus, amygdala, middle temporal and parietal gyrus as well as DLPFC. Therefore, it was labeled the "Face Emotion Processing Network" (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/brain).

Affective Working Memory Network. We identified another component that revealed a significant effect of group [$F(1,55) = 4.33$, $p = 0.04$] (Table 3), indicating that PBD and control participants engaged this network to a different degree during the fMRI task. This network included regions that are a part of the working memory circuit such as DLPFC, VLPFC, orbitofrontal cortex (OFC), medial PFC, insula, caudate, dorsal and perigenual ACC, mid-cingulate, as well as temporal and parietal regions (D'Esposito, 2007; Owen et al., 2005). This network also included the amygdala and superior temporal sulcus (STS), due to the affective and face processing components of the task (Braver et al., 2001; Passarotti et al., 2010c, 2011). We labeled this network the "Affective Working Memory Network" (Fig. 2). The regions that exhibited group differences had increased engagement to the network during the task in the overall network map, unless otherwise indicated. As illustrated in Table 4, *t*-tests in SPM5 (with AlphaSim corrected $p < 0.02$) found that PBD showed greater connectivity relative to HC in left DLPFC and caudate, and right VLPFC and medial PFC (note that the right medial PFC exhibited reduced BOLD signal activation during the task in the overall network, as indicated by downward arrows in Table 4). PBD exhibited reduced connectivity relative to HC in the junction of right DLPFC/VLPFC, amygdala, bilateral STS and left mid OFC, and precuneus, as well as in right perigenual ACC and mid-cingulate gyrus (Note that the right perigenual ACC, right midcingulate gyrus, and the left OFC exhibited reduced BOLD signal activation during the task in the overall network map, as indicated by downward arrows in Table 4).

Affect Evaluation and Regulation Network. An additional component showed a significant effect of group [$F(1,55) = 4.01$, $p = 0.05$] and of face emotion [$F(1,55) = 5.45$, $p = 0.02$], and a nonsignificant trend for a group × face emotion interaction [$F(1,55) = 2.98$, $p = 0.09$] (Table 3). The spatial network included a fronto-cingulate-temporo-parietal-limbic circuit, with regions that have been often found to be involved in evaluation of face emotion valence and in affect regulation (Brotman et al., 2010; Passarotti et al., 2010b, 2010c, 2011; Pavuluri et al., 2008). Therefore, this component was labeled the "Affect Evaluation and Regulation Network" (Fig. 3). The regions presenting with group differences had increased engagement during the task in the overall network map, unless otherwise indicated. In line with our hypotheses, two-sample *t*-tests carried out in SPM5 to examine group differences (with AlphaSim corrected $p < 0.02$) revealed that during the task relative to HC, the PBD group showed greater connectivity in this network in right dorsal ACC and bilateral medial PFC, and lesser connectivity in right VLPFC, amygdala, fusiform gyrus, putamen, posterior cingulate gyrus, bilateral insula, inferior parietal lobule, and STS (See Table 5

Table 3. Regression Coefficient Mean and Standard Deviation for β-Weights Representing Task Association for Each Network in Pediatric Bipolar Disorder and Healthy Control

| | *Time course coefficient mean (SD)* | | |
|---|---|---|---|
| | *PBD* | *HC* | *Significant effects* (p-*value*) |
| Network | | | |
| *Emotion Face Processing* | *1.252 (0.94)* | *1.681 (0.71)* | *Emotion (0.032)* |
| *Affective Working Memory* | *0.415 (0.75)* | −0.055 (0.64) | Group (0.042)<br>Group (0.049) |
| Affect Evaluation and Regulation | 0.077 (0.49) | 0.394 (0.80) | Emotion (0.023)<br>Group × emotion (0.089) |

Significant effects are reported.

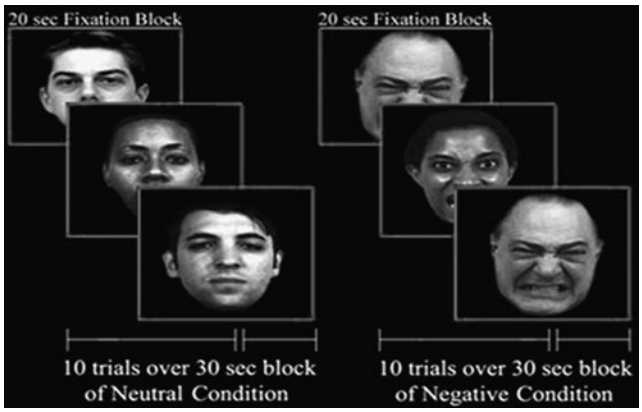


**FIG. 1.** Illustration of match trials in the two-back working memory task, with angry and neutral faces.

and Fig. 4a, b). (Note that the right posterior cingulate gyrus exhibited reduced engagement within the network during the task in the overall network map, as indicated by a downward arrow in Table 5).

*Secondary analyses*

Differential functional connectivity of the Face Emotion Processing Network for angry or neutral faces. To identify correlations between regional engagement within the face processing network and angry or neutral face blocks across groups (note that for this component, there was only a significant valence effect), we carried out *post hoc* covariate analysis in SPM5 (AlphaSim-corrected $p < 0.02$). Supplementary Table S1 details the results of the covariate analyses. To summarize, we found a positive correlation between angry face blocks and engagement of left DLPFC and precuneus, bilateral parahippocampal gyrus, and right fusiform gyrus; while there were no findings of negative correlations. For neutral faces, there was a positive correlation with left parahippocampal and fusiform gyrus, and a negative correlation with left STS. All these regions showed increased engagement during the angry and neutral face blocks in the overall network map.

Within-group differences in functional connectivity for angry or neutral faces in the Affect Evaluation and Regulation Network. To further determine within-group patterns of

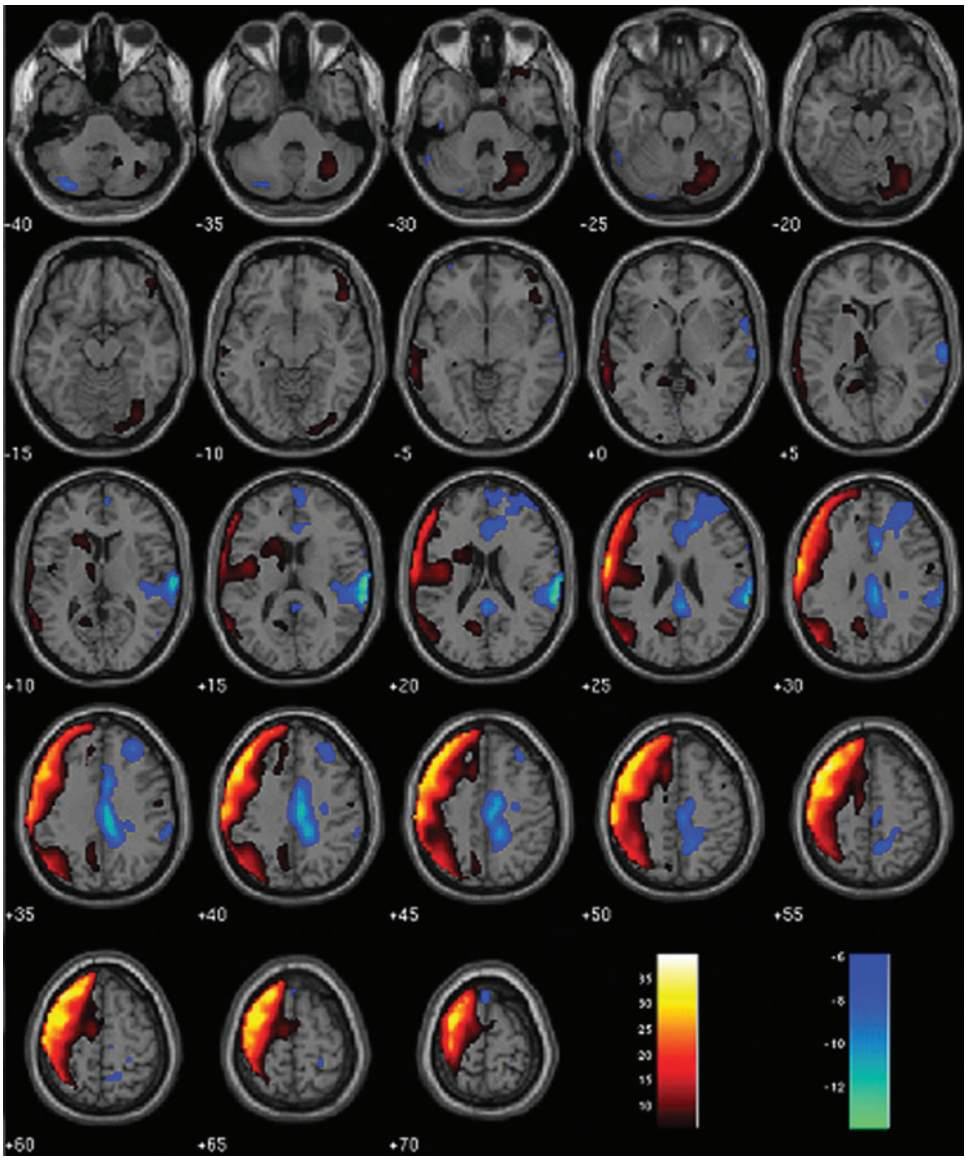


**FIG. 2.** Spatial map for functional connectivity in the Affective Working Memory Network during the affective n-back task. Within the network, red indicates increased regional engagement, and blue indicates decreased engagement, during task performance.

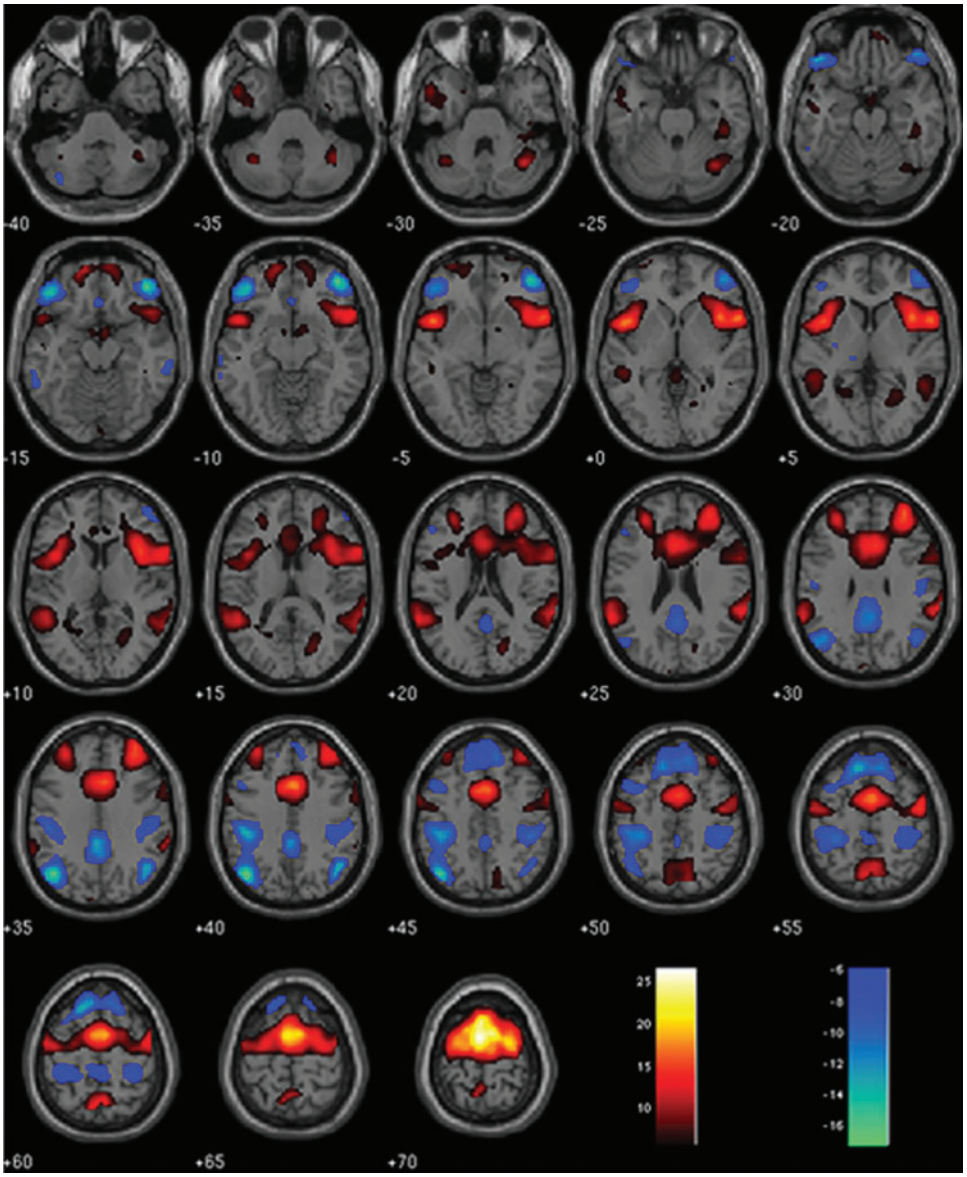


**FIG. 3.** Spatial map for functional connectivity in the Affect Evaluation and Regulation Network during task performance. Within the network, red indicates increased regional engagement during the task, while blue indicates decreased engagement during task performance.

network engagement depending on face emotion valence, we carried out a *post hoc* covariate analysis in SPM5 (AlphaSim-corrected $p < 0.02$). The main findings of this analysis are detailed in Table 6 and will be briefly summarized here. During angry face blocks, PBD exhibited a positive correlation (and, therefore, engagement during angry face blocks) in right medial OFC, bilateral insula, and posterior brain regions, and a negative correlation (and, therefore, disengagement during angry face blocks) in the right insula. In contrast, HC showed a positive correlation (i.e., engagement) in parietal regions, and a negative correlation (i.e., disengagement) in right VLPFC, bilateral ACC and insula, and left STS. For neutral face blocks, the PBD group showed a positive correlation with disengagement in left inferior parietal regions and a negative correlation with engagement in right medial OFC and parietal regions, and left dorsal ACC. HC showed a positive correlation with disengagement in left VLPFC and inferior parietal lobule, and engagement in dorsal ACC, and a negative correlation with disengagement in left STS and right posterior cingulate gyrus.

Within-group differences in temporal dynamics for angry or neutral faces in the Affect Evaluation and Regulation Network. Based on the trend for a group × face emotion interaction [$F(1,55) = 2.98$, $p = 0.09$], when examining component temporal dynamics of BOLD signal change for this network in PBD and HC separately for angry and for neutral faces, we found that in the PBD group there was no differential BOLD signal change in response to angry versus neutral faces ($p = 0.54$). In contrast, HC showed a nonsignificant trend for greater BOLD signal change for angry than for neutral faces ($p = 0.06$), with a sustained raise in BOLD signal throughout the angry face block duration, relative to neutral faces for which there was a quick BOLD signal drop off (Fig. 4c).

Correlation analyses between network functional connectivity and YMRS and CDRS scores in PBD. Detailed results for the SPM correlation analyses between clinical measures and the three networks in PBD are presented in Supplementary Table S2. We summarize results that survived Bonferroni corrections for multiple comparisons, where we used a

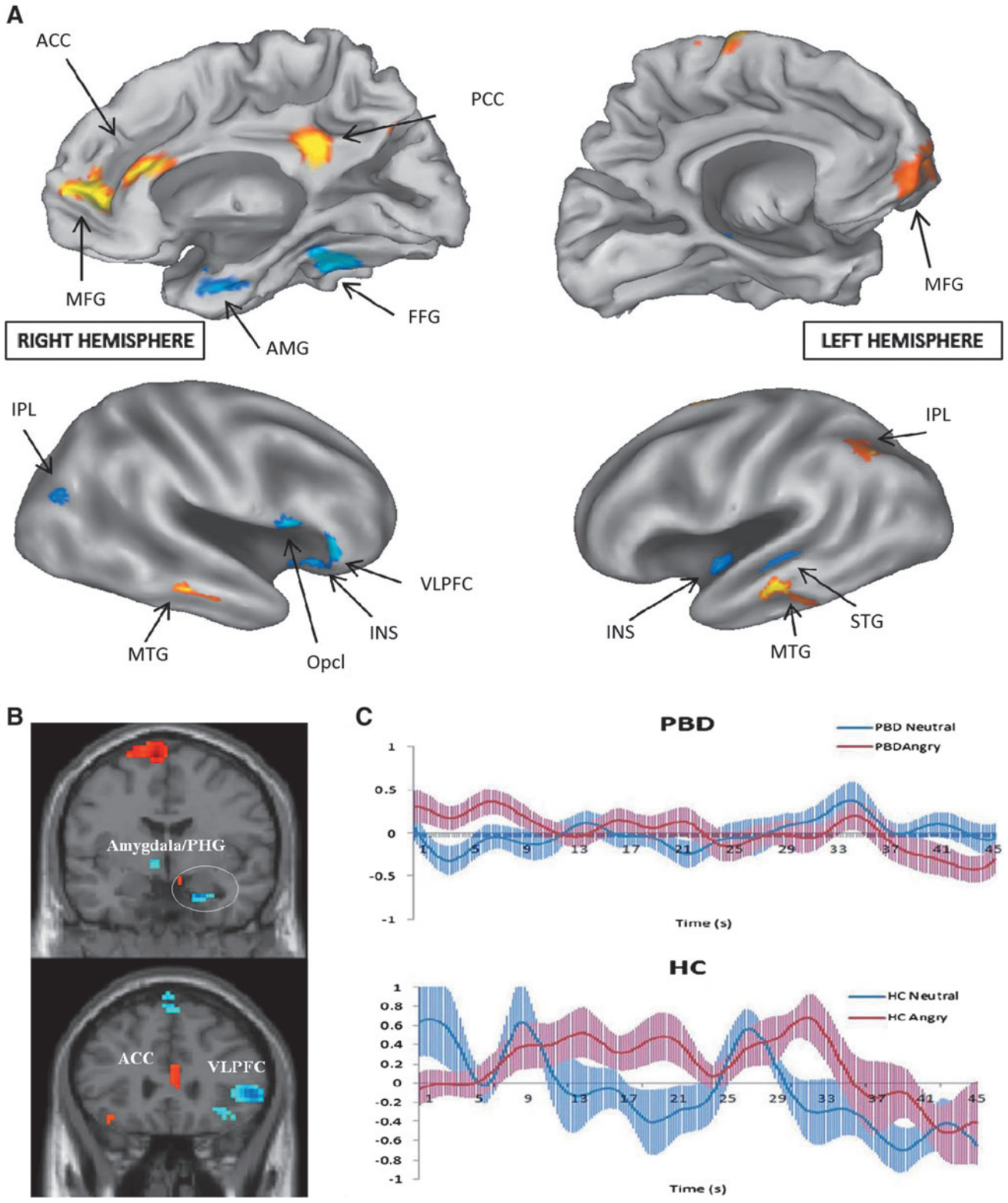


**FIG. 4.** **(A)** Between-group differences in the Affect Evaluation and Regulation Network during task performance. Yellow/orange indicates greater brain region engagement in the pediatric bipolar disorder group (PBD) than in the healthy controls group (HC): blue indicates the opposite. MFG, medial frontal gyrus; ACC, anterior cingulate cortex; pCC, posterior cingulate gyrus; AMG, amygdala; FFG, fusiform gyrus; IPL, inferior parietal lobule; MTG, middle temporal gyrus; Opcl, operculum; INS, insula; STS, superior temporal sulcus; PHG, parahippocampal gyrus. **(B)** Detail of group differences in ACC, ventrolateral prefrontal cortex (VLPFC): amygdala and PHG. **(C)** Temporal correlation fit of blood oxygenation level dependent functional magnetic resonance imaging signal change to face emotion condition (i.e., angry, neutral) in PBD and in HC. Vertical lines represent SEM.

Table 4. Between-Group Differences in Functional Connectivity for the Affective Working Memory Network

| *Brain region* | *BA* | *Peak MNI coordinates (x, y, z)* | *Voxel number* | t-*Value* |
|---|---|---|---|---|
| Greater connectivity in PBD than HC | | | | |
| ↑L DLPFC | BA 9 | −54, 21, 36 | 25 | 2.88 |
| ↑R VLPFC | BA 47/11 | 45, 36, −15 | 30 | 3.23 |
| ↑L caudate | | −12, 15, −3 | 12 | 2.61 |
| ↓R medial PFC | BA 10 | 24, 54, 18 | 177 | 3.83 |
| Lesser connectivity in PBD than HC | | | | |
| ↑R DLPFC/VLPFC | BA 45/46 | 42, 21, 21 | 51 | 3.57 |
| ↑R amygdala | | 27, 3, −21 | 12 | 2.71 |
| ↑R STS | BA 21 | 60, −33, −12 | 17 | 3.33 |
| ↑L STS | BA 21 | −60, −39, 0 | 18 | 2.71 |
| ↑L precuneus | BA 7 | −9, −51, 51 | 12 | 2.72 |
| ↓L mid OFC | BA 11 | −33, 57, −6 | 33 | 3.33 |
| ↓R perigenual ACC | BA 32 | 3, 36, 15 | 13 | 2.79 |
| ↓R mid cingulate gyrus | BA 24 | 9, −3, 36 | 20 | 2.79 |

Peak MNI coordinates and *t*-values for regions that were functionally connected within this network and which showed either greater or lesser connectivity to the network in PBD relative to HC (corrected $p < 0.02$). An upward arrow [↑] next to a specific brain region indicates increased BOLD signal change during the task in that region (i.e., positive directionality of the BOLD signal change). A downward arrow [↓] indicates decreased BOLD signal change during the task in that region (i.e., negative directionality of the BOLD signal change). DLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; VLPFC, ventrolateral prefrontal cortex; ACC, anterior cingulate cortex; STS, superior temporal sulcus; L, Left; R, Right; BOLD, blood oxygenation level dependent; MNI, Montreal Neurological Institute; BA, Brodmann area.

corrected $p < 0.01$ ($t = 2.22$). For the Affect Evaluation and Regulation Network, there was a positive correlation between YMRS scores and functional connectivity in left ventromedial PFC and right DLPFC/VLPFC junction. For the Affective Working Memory Network, there was a positive correlation between YMRS scores and functional connectivity in left DLPFC and inferior parietal lobule. Finally, for the Face Emotion Processing Network, there was a positive correlation between YMRS scores and connectivity in left STS, and a negative correlation between YMRS scores and left amygdala connectivity. With regard to the CDRS-R, for the Affect Evaluation and Regulation Network, there was a positive correlation between CDRS scores and functional connectivity of right insula and left ventromedial PFC. For the Affective Working Memory Network, there was a positive correlation between CDRS-R scores and connectivity in left inferior parietal lobule and STS, and a negative correlation between CDRS-R scores and left DLPFC connectivity. Finally, for the Face Emotion Processing Network, there was a positive correlation between CDRS-R scores and functional connectivity in right parahippocampal gyrus.

Correlation analyses between functional networks and performance measures in PBD and HC. We report only results that survived Bonferroni corrections for multiple comparisons (corrected $p < 0.002$, $t = 3.06$). In the PBD group, for the Affect Evaluation and Regulation Network, there was a positive correlation between accuracy during angry face blocks and engagement in right dorsal ACC (MNI coordinates: 7, 25, 29), and similarly, between accuracy during neutral face blocks

Table 5. Between-Group Differences in Functional Connectivity for the Affect Evaluation and Regulation Network

| *Brain region* | *BA* | *Peak MNI coordinates (x, y, z)* | *Voxel number* | t-*Value* |
|---|---|---|---|---|
| Greater connectivity in PBD than HC | | | | |
| ↑R dorsal ACC | BA 24 | 3, 26, 18 | 90 | 2.51 |
| ↑R medial PFC | BA 10 | 15, 54, 6 | 379 | 3.61 |
| ↑L medial PFC | BA 10 | −12, 66, 0 | 11 | 2.50 |
| Lesser connectivity in PBD than HC | | | | |
| ↑R VLPFC | BA 47/45 | 54, 27, 0 | 88 | 4.16 |
| ↑R insula | BA 13 | 39, 12, −9 | 105 | 3.54 |
| ↑L insula | BA 13 | −45, 9, 0 | 90 | 3.24 |
| ↑R amygdala | | 21, −3, −27 | 30 | 4.03 |
| ↑R putamen | | 21, 9, 9 | 17 | 2.98 |
| ↑R fusiform gyrus | BA 37 | 33, −51, −15 | 141 | 3.38 |
| ↑L STS | BA 21/22 | −45, −30, 3 | 158 | 4.26 |
| ↑R IPL | BA 40 | 60, −42, 27 | 26 | 3.80 |
| ↓R posterior cingulate gyrus | BA 31 | 6, −45, 33 | 14 | 2.49 |

Peak MNI coordinates and *t*-values for regions that were functionally connected within this network and which showed either greater or lesser connectivity to the network in PBD relative to HC (corrected $p < 0.02$). Upward [↑] and downward [↓] arrows indicate the same as in Table 4. Abbreviations as in Table 4. IPL, inferior parietal lobule.

Table 6. Within-Group Comparison for the Affect Evaluation and Regulation Network

| *Brain region* | *BA* | *Peak MNI coordinates (x, y, z)* | *Voxel number* | t-*Value* |
|---|---|---|---|---|
| Angry faces | | | | |
| PBD | | | | |
| Positive correlation | | | | |
| ↑R medial OFC | BA 11 | 12, 60, −15 | 45 | 3.55 |
| ↑L insula | BA 13 | −36, 6, 6 | 18 | 2.66 |
| ↑L inferior temporal gyrus | BA 20/21 | −45, −6, −36 | 26 | 3.25 |
| ↑R mid cingulate gyrus | BA 24 | 15, 9, 30 | 18 | 3.08 |
| ↑R IPL | BA 40 | 66, −30, 39 | 33 | 3.58 |
| ↓L posterior cingulate gyrus | BA 31 | 0, −57, 27 | 13 | 2.78 |
| Negative correlation | | | | |
| ↑R insula | BA 44/13 | 42, 15, 15 | 30 | 3.05 |
| HC | | | | |
| Positive correlation | | | | |
| ↑L IPL | 40 | −63, −51, 21 | 17 | 4.73 |
| ↑R precuneus | 7 | 6, −60, 48 | 94 | 4.79 |
| ↓R DLPFC | 46/10 | 51, 46, 6 | 38 | 3.82 |
| Negative correlation | | | | |
| ↑R VLPFC | BA 47 | 27, 24, −9 | 25 | 4.71 |
| ↑R dorsal ACC | BA 24 | 18, 21, 33 | 34 | 3.78 |
| ↑L dorsal ACC | BA 24 | −15, 21, 36 | 244 | 5.30 |
| ↑L Insula | BA 13 | −39, 9, −3 | 38 | 4.37 |
| ↑R insula | BA 13 | 45, 9, 0 | 18 | 4.15 |
| ↑L STS | BA 21 | −54, 0, −21 | 13 | 3.31 |
| Neutral faces | | | | |
| PBD | | | | |
| Positive correlation | | | | |
| ↓L IPL | BA 40 | −51, −63, 30 | 27 | 3.78 |
| Negative correlation | | | | |
| ↑R medial OFC | BA 11 | 9, 66, −12 | 18 | 3.69 |
| ↑L dorsal ACC | BA 32 | −3, 36, 21 | 14 | 3.17 |
| ↑R IPL | BA 40 | 60, −30, 18 | 59 | 3.29 |
| ↑R precuneus | BA 7 | 6, −60, 48 | 94 | 4.79 |
| HC | | | | |
| Positive correlation | | | | |
| ↑L dorsal ACC | BA 32 | −15, 30, 21 | 26 | 3.40 |
| ↓L VLPFC | BA 47 | −51, 39, −3 | 16 | 4.47 |
| ↓L IPL | BA 40 | −45, −39, 39 | 111 | 3.65 |
| Negative correlation | | | | |
| ↓L STS | BA 21 | −60, −39 −18 | 22 | 4.11 |
| ↓R posterior cingulate gyrus | BA 31 | 12, −42, 30 | 17 | 3.80 |

MNI coordinates and *t*-values for regions where functional connectivity was positively or negatively correlated with angry or happy face emotions in PBD and in HC. Upward [↑] and downward [↓] arrows indicate the same as in Table 4. Abbreviations as for Table 4.

and engagement in right mid-cingulate gyrus (MNI: 1, 0, 27). In the same network, for HC, there was a positive correlation between accuracy during angry face blocks and engagement in right insula (MNI: 36, 8, −5), and between accuracy during neutral face blocks and engagement in right VLPFC (MNI: 45, 24, 4). There were no significant results for RT.

## Discussion

Using ICA methodology, we identified temporally independent and distributed brain networks corresponding to well-described circuitry related to face processing (Haxby et al., 2002; Passarotti et al., 2007), affect regulation (Passarotti et al., 2010a, 2010c; Pavuluri et al., 2008; Rich et al., 2006), and working memory processes (Owen et al., 2005; Passarotti et al., 2010a, 2010c). The primary study finding was that while there were no group differences for the Face Processing Network, the PBD patients showed altered functional integration of amygdala, medial PFC, and VLPFC relative to HC within both the Affective Working Memory and the Affect Evaluation and Regulation Network. Functioning in these regions has been found to be abnormal in several fMRI studies conducted on PBD (Leibenluft et al., 2007; Passarotti et al., 2010a, 2010b, 2010c, 2011; Pavuluri et al., 2008) as well as some functional connectivity studies in adult BD (Foland et al., 2008). Secondary behavioral findings revealed that in PBD, performance accuracy was positively correlated with engagement in cingulate cortex regions; whereas in HC, performance accuracy was positively correlated with engagement in right insula and VLPFC, which is possibly an indication of differential regional engagement for task performance in the two groups.

### *PBD dysfunction in the Affective Working Memory Network*

The main finding for the Affective Working Memory Network was that the PBD group relative to HC showed greater connectivity in prefrontal and striatal regions typically involved in working memory function (i.e., left DLPFC, right VLPFC, and left caudate) (Owen et al., 2005), which may be due to increased cognitive effort (Dosenbach et al., 2008). Moreover, relative to HC, the PBD group showed decreased engagement in the right amygdala and STS, which are regions typically involved in face and affect processing, as is required in the current task, as well as in the DLPFC/VLPFC junction, with integrated cognitive and affective processes (Passarotti et al., 2010a, 2011; Pavuluri et al., 2008; Petrides and Pandya, 2002), and in parietal regions associated with attentional vigilance and rehearsal processes during working memory tasks (Owen et al., 2005). Our ICA findings of reduced functional connectivity in these regions are in line with our previous PBD studies using conventional fMRI analyses of the BOLD signal, which showed reduced hemodynamic response in PBD relative to HC in control regions at the interface of cognition and affect (Passarotti et al., 2010b, 2010c; Pavuluri et al., 2008). In sum, our present data suggest that within this network, the PBD group exhibits increased engagement of fronto-striatal working memory regions relative to HC, possibly due to cognitive effort, coupled with reduced functional connectivity in regions involved in affect processing and in the integration of affect and working memory processes.

### *PBD dysfunction in the Affect Evaluation and Regulation Network*

The present results for the Affect Evaluation and Regulation Network confirm our hypotheses and are in line with previous fMRI findings in PBD (Passarotti and Pavuluri, 2011; Passarotti et al., 2010a, 2010b, 2010c, 2011; Pavuluri et al., 2008), or functional connectivity findings in adult BD (Almeida et al., 2009; Foland et al., 2008), indicating dysfunction in key regions for emotion processing, evaluation, and regulation. Specifically, PBD showed reduced connectivity relative to HC in right VLPFC, amygdala and bilateral insula, and temporo-parietal regions. The ability to regulate emotions relies on efficient communication between a distributed network of regions that are involved in bottom-up emotional perception and in top-down cognitive evaluation of emotions (Passarotti and Pavuluri, 2011). Specifically, the integration of input from amygdala and insula with processing in the OFC and VLPFC is essential for appropriate prefrontal evaluation of emotional information and consequent regulation of affect (Chang et al., 2004; Passarotti et al., 2011; Pavuluri et al., 2008). While further investigations are needed, the present results suggest that the compromised ability to process and regulate emotions in PBD may be related to a reduced functional connectivity, and presumed reduced neural communication, between these regions.

It should also be noted that within this network, the PBD group showed increased functional connectivity relative to HC in right dorsal ACC and in bilateral medial PFC. Recently, these regions have been found to be a part of a neural subcircuit involved in "rumination," a process with recursive self-focused thinking that leads to worsening of negative mood (Cooney et al., 2010). Rumination processes may be associated with both mania and depression in BD (Johnson et al., 2008). Abnormal medial PFC connectivity emerged during a resting-state study using ICA in BD (Ongür et al., 2010). Moreover, a functional connectivity study with depressed adults (Sheline et al., 2010) identified a common over-active medial PFC region that was suggested to "hot wire" three different brain networks, for cognitive control, default mode, and affect, leading to excessive self-focus, increased vigilance, and emotional, visceral, and autonomic dysregulation. While the present study did not have any direct measure of rumination that could be directly correlated with medial PFC connectivity, the significant correlation which we found, for the Affect Evaluation and Regulation Network, between YMRS and CDRS scores and engagement in left ventromedial PFC in the PBD group, tentatively points at a role of this prefrontal region in manic and depressive symptoms that may be related to greater self-focused thinking or rumination.

Finally, an important finding for this network resulted from an exploratory secondary analysis in which we further examined differential network engagement while processing angry and neutral faces within each group. Interestingly, in PBD there was a positive correlation between the engagement of the insula, ventromedial, and posterior brain regions involved in this same "rumination subcircuit" (Cooney et al., 2010) and measurements of how strongly this network was engaged during the angry face block. In contrast, for neutral faces, PBD showed a negative correlation with OFC, dorsal ACC, and parietal regions. This altered prefrontal region engagement for both the neutral and angry face condition in PBD, which is in contrast to the HC pattern of only negative correlation with right prefrontal regions for the angry faces, suggests a generalized challenge for prefrontal regulatory regions in PBD. In line with this pattern of results, when examining component temporal dynamics for this network, we also found that the BOLD response did not differ in PBD based on face emotion condition. On the contrary, we found condition-based BOLD activation in HC, with greater engagement for angry faces possibly because of increased attentional arousal with negative emotion (Williams et al., 1996). These findings, therefore, suggest that even neutral faces may be seen as emotional or potentially threatening in PBD, leading to increased self-focus on the patient's own internal state (Rich et al., 2006).

In sum, the present results indicate that, in the PBD group, amygdala, insula, and prefrontal regulatory regions are more latent or disengaged relative to the rest of the affect regulation network; while regions involved in self-focused thinking are over-engaged, possibly leading to inefficient affect regulation that is associated with manic and depressive symptoms.

### *The amygdala exhibits decreased functional connectivity within the Affect Regulation Network and the Working Memory Network in PBD*

The PBD group showed reduced functional connectivity in right amygdala relative to HC both for the Affect Evaluation and Regulation and for the Affective Working Memory Network. An increased hemodynamic response in the amygdala is often found in adult (Foland et al., 2008) and child (Passarotti et al., 2011; Pavuluri et al., 2008; Rich et al., 2006) BD populations, and amygdala over-reactivity to emotional stimuli is interpreted as contributing to the persistent affect dysregulation in BD. Moreover, emotion perception is the result of both

bottom-up and top-down processes (Ochsner et al., 2009). A previous fMRI study using the same affective working memory paradigm found increased right amygdala activation in PBD relative to HC, which correlated with YMRS scores. In light of the evidence that the right amygdala is involved in automatic bottom-up emotion generation processes (Ochsner et al., 2009), and may be particularly involved in emotional face processing (Passarotti et al., 2011; Pavuluri et al., 2008), the present data suggest that reduced functional connectivity in right amygdala may lead to altered communication of emotional information to prefrontal regions such as the medial OFC and VLPFC, which are involved in more cognitive, or top-down, emotion evaluation and regulation processes (Ochsner et al., 2009).

The present results are in line with initial evidence of reduced functional connectivity between amygdala and VLPFC (Chepenik et al., 2010; Foland et al., 2008) or perigenual ACC (Wang et al., 2009) in adults with BD, and between the amygdala and temporal association regions implicated in processing facial expressions and social stimuli in PBD (Rich et al., 2008). Our findings suggest a potential mechanism for amygdala dysfunction in PBD by revealing that while the amygdala is often over-reactive to emotional stimuli in PBD, it is, nevertheless, de-synchronized relative to the concerted engagement of other prefrontal and posterior brain regions involved in affect evaluation and regulation. This initial interpretation warrants future studies that incorporate concurrent fMRI and ICA analyses, to directly assess whether during emotional challenge, amygdala dysfunction manifests itself both in terms of its reactivity to emotional stimulation, which can be examined by looking at changes in BOLD signal activation, and in terms of its influence on emotion evaluation and regulation processes, which can be examined in terms of its functional connectivity to affective and cognitive networks. Finally, correlation analyses revealed that within the Face Emotion Processing Network, the lower the functional connectivity was in the left amygdala, the more severe the manic symptoms were in PBD, confirming a direct relationship between amygdala dysfunction and manic symptoms that may relate to face emotion processing.

### *Differential abnormal connectivity patterns in VLPFC in the affect regulation and the Working Memory Network in PBD*

This functional connectivity study is among the first that shows the concurrent involvement of the VLPFC in different networks, and divergent connectivity patterns in the VLPFC depending on the network considered, in PBD. Our functional connectivity findings are in line with fMRI evidence that the VLPFC is a heteromodal region, or a "hub" which is involved in a number of distinct though overlapping processes, such as emotion regulation (Passarotti and Pavuluri, 2011; Pavuluri et al., 2008), attentional maintenance of stimuli during working memory performance (Owen et al., 2005), and inhibition during cognitive (Aaron et al., 2003; Passarotti et al., 2010b; Pavuluri et al., 2010) and affect (Passarotti et al., 2010a, 2010b, 2010c, 2011; Pavuluri et al., 2008) processing. Importantly for our hypotheses, we found that depending on the functional network considered, the functional connectivity in VLPFC was either decreased or increased in PBD patients relative to HC. For the Affect Evaluation and Regulation Network relative to HC, PBD exhibited reduced functional connectivity to the network in a VLPFC region (BA 45/47) that is involved in both inhibition and affect regulation (Aaron et al., 2003; Passarotti et al., 2010a, 2010b; Pavuluri et al., 2008). This region also exhibited reduced hemodynamic response in PBD in previous fMRI studies during a response inhibition task (left VLPFC, BA 47/10) (Passarotti et al., 2010b) and an affective stroop-like task (right VLPFC, BA 47/10) (Passarotti et al., 2010a). Therefore, these findings in PBD suggest reduced affect regulation capacity in VLPFC, both in terms of its hemodynamic activation and of its functional integration. On the other hand, for the Affective Working Memory Network, PBD showed increased functional connectivity in a more inferior and orbital region (BA 47/11), possibly suggesting increased effort in face emotion processing and evaluation during the working memory task (Wager et al., 2008).

The VLPFC has been found to be functionally (Drevets et al., 2008; Leibenluft et al., 2007; Passarotti et al., 2010a, 2010b; Pavuluri et al., 2008; 2009a) and structurally (Bora et al., 2010) abnormal in BD and in unaffected relatives (McDonald et al., 2004), and has, therefore, been proposed as a candidate bio-marker in BD pathophysiology (Foland et al., 2008). In view of the present findings, future studies will need to further investigate different VLPFC contributions to segregated functional networks that operate at the interface of cognition and affect to better characterize this region as a bio-marker in PBD.

This study has some limitations that suggest caution in interpreting the present results. First, while a block design offers greater statistical power and BOLD signal stability relative to an event-related design, it cannot address specific neural alterations resulting from correct or incorrect responses, which would require an event-related design. Second, in our experimental protocol, we included a 20 sec fixation in between blocks to allow for emotional arousal to return to baseline. However, we do not have any direct evidence that emotional arousal, in fact, returns to baseline within this time interval in both groups, or whether in patients this time may be prolonged, and, therefore, this aspect may have affected the functional connectivity differences which we found between PBD and HC. Third, we cannot exclude that group differences in performance, and not just diagnosis, may be responsible for the present group differences in network functional activation. Fourth, the PBD sample was larger than the HC sample. Although there were no significant group differences in the components considered when we tested for equality of variance, it will be important that future studies on functional connectivity use comparable sample size to avoid any potential problem. Fifth, the current study does not account for potential group differences in baseline physiological conditions, such as baseline cerebral blood flow, which could potentially affect the results. Therefore, interpretation of our findings should be cautious. Finally, while ICA methodologies were ideal for our initial goal to examine group differences in distributed neural networks, future studies will need to address questions related to "effective" connectivity, and to further examine the abnormal functional connectivity between prefrontal regions involved in emotion appraisal and regulation and limbic regions that contribute to persistent mood dysregulation in PBD.

## Conclusions

This is the first whole-brain functional connectivity study conducted on functional networks underlying the interaction

between affective and working memory systems in PBD. The present functional connectivity results suggest that PBD patients exhibited greater engagement when evaluating facial emotion and performing working memory processes, and reduced engagement in regions supporting affect regulation. Our findings of reduced functional connectivity in prefrontal regions and amygdala in PBD relative to HC may be potential bio-signatures of neural dysfunction in PBD.

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### Author Disclosure Statement

Dr. Passarotti and the coauthors have no competing financial interests to declare.

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