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ABSTRACT

A major paradigm shift in mental health has led to the ascendance of the view that chronic psychopathology results from perturbed neural development. While most work in this area examines schizophrenia, the current report extends the paradigm to bipolar disorder (BD) in youth, thus demonstrating traction (not sure I understand what you mean here) in the developmental-psychobiology perspective. To study the role of amygdala dysfunction, we examined the neural mechanisms mediating face processing in 22 youth (mean age 14.21 $\pm$ 3.11 years) with BD and 21 controls of comparable age, gender, and IQ. Event-related fMRI compared neural activation when attention was directed to emotional aspects of faces (hostility, subjects' own fearfulness) vs. non-emotional aspects (nose width). Compared to controls, patients perceived greater hostility in neutral faces, and reported more fear when viewing them. Also, compared to controls, patients had greater activation in the left amygdala, accumbens, putamen, and ventral prefrontal cortex when rating face hostility, and greater activation in the left amygdala and bilateral accumbens when rating their own fear of the face. There were no between-group behavioral or neural differences in the non-emotional conditions. Results implicate deficient emotion-attention interactions in the pathophysiology of BD in youth, and suggest that developmental psychobiology approaches to chronic mental illness have broad applicability.

INTRODUCTION

Recently, psychology and psychiatry have witnessed a major paradigm shift, in that virtually all chronic adult mental illnesses are now viewed as end results of long-term perturbations in the development of specific neural circuits. Two lines of research support such a developmental perspective: family-based/longitudinal and neurobiological studies. Family-based and longitudinal studies provide strong support for developmental perspectives in a range of conditions, including behavior disorders, substance abuse, mood disorders, and the psychoses (1-3). However, virtually all research on developmental neurobiology focuses on schizophrenia, where the dominant theories implicate a neural circuit connecting the dorsolateral prefrontal cortex to the striatum and hippocampus (4, 5). Thus, an important next step in this developmentally focused paradigm shift is the extension of the neurobiological research approach to other mental illnesses.

For several reasons, bipolar disorder (BD) is an ideal illness in which to conduct neurobiologically-oriented developmental research. The disorder is highly impairing, causing marked disruption in social, academic, and family functioning. Major questions persist concerning the boundaries of the condition in children, and neurobiological data might ultimately speak to this controversy. Most importantly, research in adult patients and animals implicates a circuit encompassing the amygdala, striatum, and associated areas of the ventral prefrontal cortex (VPFC) in the pathophysiology of BD (6). This circuit has been shown to have considerable developmental plasticity. In particular, the identification of amygdala-based perturbations in children with BD would have profound implications for developmental conceptualizations of chronic mental illness, since it

would suggest that perturbations in neural development play a role in diverse mental illnesses, with specific circuits implicated in specific conditions.

Research to date supports the value of developmental studies focusing specifically on amygdala structure and function in BD. Structural magnetic resonance imaging (MRI) studies in bipolar adults find either increased or unchanged amygdala volume relative to controls (7-11), while studies in bipolar children consistently document decreased amygdala volume in patients compared to controls (12-16). fMRI studies find that adults with BD, relative to controls, have either amygdala hyperactivation (17) or hypoactivation in response to facial stimuli (18). While the few fMRI studies in pediatric BD have not revealed functional abnormalities in the amygdala (19, 20), these studies have not employed paradigms ideally suited for examining amygdala function.

Here, we used an fMRI face processing task to study youth with BD and controls. We link behavioral measures of emotion processing with function in a neural circuit encompassing the amygdala, VPFC, and striatum, using a task that directs subjects' attention toward or away from emotional aspects of faces rated neutral by a healthy, normative sample. We chose this paradigm because children with BD have difficulty categorizing facial emotions (21), and their attentional performance is more impaired in emotional than in non-emotional contexts (22). These two deficits may be related: children with BD may mislabel facial emotions because their affective response to a face disrupts emotion categorization. We also chose this paradigm because it engages the amygdala-striatal-VPFC circuit (23, 24), which has been implicated in the pathophysiology of BD, and mediates facial expression processing and the direction of attention toward or away from emotional stimuli (25-29). We used neutral faces in the

task because individuals with mood disorders tend to misperceive such faces as negative (30, 31). Moreover, the current study was able to document the on-line occurrence of such cognitive misperceptions in BD during face viewing, allowing us to elucidate amygdala-based correlates of this deficit.

Consistent with previous research (22), we expected to see behavioral and neurophysiological deficits in BD youth only when they attended to emotional aspects of the neutral face. Specifically, we hypothesized that during emotional, but not non-emotional tasks, children with BD, compared to controls, would have greater activation in the amygdala, striatum, and VPFC, and would report more negative subjective ratings and have slower reaction times.

MATERIALS AND METHODS

Inclusion/Exclusion Criteria

BD (N=22) and control (N=21) subjects were enrolled in an ongoing neurocognitive and neuroimaging study at the National Institute of Mental Health (NIMH). Subjects were recruited via advertisements to patient advocacy groups and letters to practicing psychiatrists. The NIMH IRB approved the study. Parents and children gave written informed consent/assent.

Inclusion criteria for the BD sample required that subjects ages 9-17 meet Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)(32) criteria for BD, including a history of at least one hypomanic or manic episode meeting full duration criteria—i.e. lasting $\geq$ 4 days—during which the child exhibited abnormally elevated and expansive mood and at least three other criterion "B" symptoms (33).

Children with a history of irritability only, without elevated or expansive mood, and those without distinct manic episodes were excluded; thus, this sample met criteria for the narrow phenotype of pediatric BD (34). The Kiddie-Schedule for Affective Disorders-Present and Lifetime Version (K-SADS-PL) (35) was administered to parents and children separately by clinicians with established reliability (i.e. kappa $\geq$ 0.9). Comorbid psychiatric diagnoses were based on symptoms present during euthymia. Control subjects and a parent also completed the K-SADS to ensure that the subject had no psychiatric history.

Exclusion criteria included I.Q. < 70, pervasive developmental disorder, psychosis that interfered with study compliance, unstable medical illness, substance abuse within two months of the initial evaluation, and for controls, psychiatric illness in a first-degree relative.

To evaluate mood at the time of scanning, graduate level clinicians with established inter-rater reliability administered to patients and their parents the Children's Depression Rating Scale (CDRS)(36), and the Young Mania Rating Scale (YMRS)(37) within 24 hours of the scan. IQ was measured with the Wechsler Abbreviated Scale of Intelligence (WASI)(38).

Behavioral Task

Participants viewed 32 adult faces (8 each of happy, angry, fearful, and neutral). Grayscale face stimuli were selected from standardized sets constructed by Ekman and Friesen (39), Gur (www.uphs.upenn.edu/bbl/pubs/downloads/nptasks.shtml), and Tottenham and Nelson (www.macbrain.org/faces/index.htm). As previously noted, in this

analysis we included only neutral face trials in order to maximize our ability to detect between-group differences in emotion-attention interactions. However, subjects were also shown emotional faces (i.e. happy, angry, fearful) to reduce boredom and habituation from repeated exposure to similar stimuli (40, 41).

We used the frequently employed "rapid event related" paradigm of Friston (42) and Zarahn & Slifstein (43). On each trial, using a 5-key button box (MRI Devices, Waukesha, WI), subjects rated the displayed face on one of three 5-point scales: 1) the threat-level of the face ("How hostile is the face?"); 2) their emotional response to the face ("How afraid are you?"); or 3) a non-emotional facial feature ("How wide is the nose?"). These rating tasks directed the subject's attention to either emotional or non-emotional aspects of the face. Fixation trials were also included as control trials and to facilitate data analysis. Each face or fixation cross was displayed for 4000 ms to allow subjects to rate each face while viewing it, with minimal demands on working memory (44). Each stimulus or fixation was followed by an inter-trial interval varying in duration from 750-1250 ms. Visual stimuli were displayed on Avotec Silent Vision Glasses (Stuart, FL). Practice sessions with novel stimuli ensured that participants understood the tasks before scanning.

There were four blocks in the experiment: one for each of the three rating types, and one for passive viewing. Rating instructions were presented for 3000 ms before each block. Each block comprised 10 trials (eight of the 32 faces, 2 fixations). Blocks were grouped into epochs, with each epoch consisting of four blocks, one for each task. These four 40-trial epochs were integrated into one 160-trial run. Block and trial order were randomized across participants.

MRI Data Acquisition

Whole-brain blood oxygen level dependent (BOLD) fMRI data were acquired on a General Electric Signa 3T scanner. Following sagittal localization and manual shimming, functional T2* weighted images were acquired using an echo-planar single-shot gradient echo pulse sequence with a matrix size of 64 x 64, repetition time (TR) of 2000 ms, echo time (TE) of 40 ms, field of view of 240 mm and voxels of 3.75 x 3.75 x 5 mm. Images were acquired in 23 contiguous 5 mm axial slices per brain volume positioned parallel to the AC-PC line. All functional data were gathered in a single 14 minute run for each subject. After EPI acquisition, a high resolution T1 weighted anatomical image was acquired to aid with spatial normalization. A standardized magnetization prepared gradient echo sequence (180 1 mm sagittal slices, FOV=256, NEX=1, TR=11.4 ms, TE=4.4 ms, matrix=256x256, TI=300 ms, bandwidth=130 Hz/pixel, 22 kHz/256 pixels) was used.

Data Analysis

Behavioral Data

To evaluate potential between-group differences in behavioral performance, two 2 x 3 repeated measures ANOVAs were conducted. Dependent variables for the two ANOVAs were rating scores (hostility, afraid, nose width) and reaction time (RT), respectively. For both ANOVAs, group (BD vs. control) was the between-subject factor, and rating type (hostility, afraid, nose width) was the within-subject factor. Statistical corrections were implemented where assumptions of sphericity or homoscedasticity were

violated. The Greenhouse-Geisser procedure was applied when appropriate. Post-hoc comparisons employed the Tukey test.

fMRI Data

For each subject, reconstructed fMRI images were examined for excessive motion using MedX software. Subjects who moved more than 1.5 mm in any plane were discarded. Analyses were conducted with SPM software (SPM99, Wellcome Department of Neurology, London U.K.) and Matlab 5.3 routines. Functional data were corrected for slice timing, motion corrected, co-registered to the anatomical data, and spatially normalized to a Montreal Neurological Institute (MNI) T1-weighted template image supplied with SPM99. After preprocessing, fMRI images were visually inspected to evaluate the quality of the normalization procedure.

At the individual subject level, event-related response amplitudes were estimated using the General Linear Model (GLM) for each of the four event types: subjects rating afraid, hostility, or nose width during neutral face viewing, and fixation. The waveform used to model event-related response in the GLM was a rectangular pulse (4s duration) convolved with the hemodynamic response function specified by SPM99. Contrast images were generated for each subject using pairwise comparisons of the event-related BOLD responses across event types. Since the goal of the current study was to examine emotion-attention interactions, the contrasts of interest compared activation during an emotional task (rating hostility or fear) vs. a non-emotional task (rating nose width or viewing a fixation cross). Thus, the primary contrasts, all with neutral faces, were: afraid rating vs. nose width rating (“afraid vs. nose”), afraid rating vs. fixation (“afraid vs.

fixation"), hostile rating vs. nose width rating ("hostile vs. nose"), and hostile rating vs. fixation ("hostile vs. fixation").

Each contrast image was divided by the subject specific voxel time series means, yielding values proportional to percentage fMRI signal change (45). Each contrast image was then smoothed with an isotropic Gaussian kernel (FWHM =11.4) to decrease non-stationarity in the spatial autocorrelation structure introduced by the previous step.

For all group-level analyses, a random effects model was used to permit population-level inferences (46). To test our *a priori* hypotheses, we used the Gaussian random field threshold ($p<.05$) in selected regions of interest (ROIs) and applied the small volume correction within each region. The ROIs were bilateral amygdala, VPFC, and ventral striatum (i.e. accumbens, putamen, and caudate). Each was defined, using standard anatomical criteria (47), on the canonical structural MRI images provided by SPM 99 software and then applied to all normalized brains at the group level. Coordinates are in Montreal Neurological Institute (MNI) space. Binary masks for each ROI consisted of voxels in which all subjects showed a measurable BOLD response; thus subjects' data were not differentially affected by signal dropout in difficult-to-image regions.

Although all faces were shown for 4 seconds, between-group differences in RT or ratings may reflect between-group differences in the time spent attending to the face or evaluating emotional aspects of the stimuli; these could affect the magnitude of the BOLD signal (48). Thus, secondary analyses covaried for RT and ratings in instances where neural activation differed significantly between patients and controls. Bivariate correlational analyses were used to examine associations between the magnitude of the

BOLD signal at the peak voxels within those ROIs that showed significant between-group differences, and ratings of hostility or fear.

RESULTS

Participant Demographics and Clinical Characteristics (Supporting Text: Table 1)

Patients (N = 22) and controls (N = 21) did not differ on age (BD=14.2 ± 3.1 yrs; control= 4.5 ± 2.5 yrs), sex (male: BD=45.5%; control=52.4%), or IQ (BD=109.3 ± 11.6; control=114.3 + 11.4). Of the patients, 90.9% (N=20) met criteria for Bipolar I; 81.8% (N=18) had at least one additional diagnosis, and the mean number of comorbid diagnoses was 1.4 ± 1.1. At the time of scanning, 81.8% (N = 18) of patients were medicated, with a mean of 2.5 ± 1.8 medications per subject.

The patients' mean CDRS score was 29.2 ± 9.3, and the mean YMRS score was 9.0 ± 6.1. 54.5 % (N=12) of the children with BD were euthymic at the time of scanning. Of the ten non-euthymic patients, four were depressed (CDRS > 40, YMRS <12) and six were hypomanic (YMRS > 12 but < 26, CDRS < 40).

Behavioral Data

Ratings

The repeated measures ANOVA showed a significant group x ratings interaction [$F(2,82)=6.08$, $p=.008$)]. Post hoc analyses found that patients, compared to controls, rated the neutral faces as significantly more hostile (BD=2.00 ± 0.61; controls=1.56 ± 0.39; $t=2.80$, $p=.008$) and themselves as significantly more afraid (BD= 2.02 ± 0.88;

control=1.39 ± 0.38; t=3.02, p=.004) (Supporting Text: Table 2a). There were no between-group differences on nose width ratings.

Reaction time

The repeated measures ANOVA of RT during ratings found a significant group x ratings interaction [F(2,40)=11.36, p<.001)]. Post hoc analyses found that patients were significantly slower than controls to rate the faces' hostility (BD=2203.59 ± 326.12 ms, controls= 1754.90 ± 276.08 ms; t=4.86, p<.001) (Supporting Text: Table 2b). There were no significant between-group differences in RT on afraid or nose width ratings.

fMRI Data

Primary between-group contrasts

Afraid vs. nose width; afraid vs. fixation

We compared patients and controls on neural activation when viewing neutral faces and rating "how afraid" vs. "nose width" (i.e., the "afraid vs. nose width" contrast). Compared to controls, patients had significantly greater activation in the left amygdala (t=4.05, p=.001) and bilateral accumbens (left: t=3.68, p=.003; right: t=2.58, p=.037) (Figure 1; Supporting Text: Table 3). Similarly, on the afraid vs. fixation contrast, patients had significantly greater activation than controls in the bilateral amygdala (left: t=3.40, p=.008; right: t=3.03, p=.019), left accumbens (t=3.04, p=.018), and left putamen (t=3.45, p=.016) (Supporting Text: Table 3).

Hostile vs. nose width; hostile vs. fixation

Activation (to neutral faces?) during the hostility rating and the two non-emotional tasks was compared. In the hostile vs. nose width contrast, patients had significantly greater activation than controls in the left amygdala (t=3.44, p=.006), left accumbens (t=2.81, p=.025), left putamen (t=3.13, p=.026), and left VPFC (t=3.21, p=.032) (Figure 2; Supporting Text: Table 3). Similarly, in the hostile vs. fixation contrast, patients had significantly greater activation than controls in the bilateral amygdala (left: t=3.22, p=.011; right: t=2.93, p=.021) and bilateral putamen (left: t=3.11, p=.029; right: t=2.87, p=.049) (Supporting Text: Table 3).

Post hoc contrasts

Within-group contrasts on emotional tasks

To confirm that the between-group differences reflected limbic hyperactivation during the emotional tasks in patients but not controls, we performed within-group comparisons. In patients, the afraid vs. nose width contrast revealed significant activation in the left amygdala (t=4.32, p=.001), left nucleus accumbens (t=2.76, p=.028), and bilateral VPFC (left: t=3.87, p=.007; right: t=2.90, p=.05) (Supporting Text: Table 4). A similar analysis in controls revealed activation only in the right VPFC (t=3.64, p=.01). In the hostile vs. nose width contrast, patients had activation in the bilateral amygdala (left: t=4.09, p=.001; right: t=2.57, p=.04), left accumbens (t=2.81, p=.025), left caudate (t=2.98, p=.031), and left putamen (t=2.98, p=.036) (Supporting Text: Table 4). The same contrast in controls revealed no activation.

Between-group contrasts on non-emotional tasks

To ascertain if neural activation differed between the two groups on the non-emotional control tasks, we compared patients and controls on the nose width rating vs. fixation contrast. There were no between-group differences on this contrast.

Associations with behavioral data

Covarying for reaction time and ratings

Because patients were significantly slower than controls to make hostility ratings, we examined the hostile vs. nose width and hostile vs. fixation contrasts using RT as a covariate. All between-group differences remained significant. Specifically, patients had significantly greater activation than controls in the left amygdala ($t=3.71$, $p=.003$), accumbens ($t=2.54$, $p=.044$), and putamen ($t=2.86$, $p=.047$) on the hostile vs. nose width contrast, and in the left amygdala ($t=2.47$, $p=.05$) and putamen ($t=2.80$, $p=.05$) in the hostile vs. fixation contrast.

Given that our patients rated neutral faces as significantly more hostile and fear-provoking than did controls, we re-examined the contrasts using ratings as a covariate. All of the ROIs that showed significant between-group differences in the afraid vs. nose width and afraid vs. fixation contrasts remained significant when controlling for ratings. On the hostile vs. nose width contrast, the left amygdala ($t=3.44$, $p=.006$) and VPFC ($t=3.21$, $p=.032$) remained significant when covaried for ratings, and on the hostile vs. fixation contrast, the bilateral amygdala (left: $t=3.22$, $p=.011$; right: $t=2.93$, $p=.021$) difference remained significant.

Correlation of neural activation with ratings

Because patients rated the faces as more hostile and fear-producing than did controls, for each group we examined correlations between ratings and activation at the peak voxel of each ROI that had revealed significant between-group differences. In controls, there were no significant correlations between ratings and activation; in the patients, hostile ratings correlated with activation of the left amygdala on the hostile vs. nose width rating ($r=.51$, $p=.01$). Between-group comparison of the correlation between left amygdala activation and the hostile vs. nose width rating within each sample using a Fisher r-to-z calculation found the correlation in the BD sample to be significantly greater than that in controls ($z=2.32$, $p=.02$) (Supporting Text: Figure 3).

Relationships between mood, medication, comorbidity, and activation

Given the heterogeneity of our patients with regard to mood status during scanning, comorbid diagnoses, and treatment, we conducted a series of ANOVAs and bivariate correlational analyses to compare activation within subgroups of the patients at the peak voxels in the ROIs. We found no differences in activation between euthymic and non-euthymic patients, those with and without comorbid anxiety disorders, or those with and without comorbid ADHD. Also, there were no significant correlations between neural activation and CDRS or YMRS scores. Finally, we found no significant correlations between activation and the number of medications patients were taking. Moreover, examination of fMRI response in the four medication-free subjects revealed similar response patterns in medicated and un-medicated subjects. Nevertheless, given the small sample sizes in these post-hoc analyses, one must interpret them cautiously.

DISCUSSION

An emerging paradigm views chronic mental illnesses as developmental perturbations in specific neural circuits. Prior studies implicate a DLPFC-based circuit in developmental models of schizophrenia and an amygdala-based circuit in adult BD samples. Our results in pediatric BD are consistent with the latter work: we demonstrate functional deficits in an amygdala-striatal-VPFC circuit (49), while demonstrating that such hyperactivation occurs specifically when patients attend to the emotion that they perceive on a face, or to their emotional response to a face, but not when they attend to a non-emotional facial feature.

Taken together, prior studies in schizophrenia and the current study in pediatric BD document the broad applicability of the developmental psychobiology approach. Diverse forms of chronic psychopathologies that typically manifest in adulthood appear to reflect the end result of developmental perturbations in psychobiology. Furthermore, distinct conditions involve perturbations in distinct neural circuits. The hope is that the identification of psychobiological abnormalities early in the course of an illness may allow for more successful interventions before a chronic course is established.

Given that we were able to specify the context in which limbic dysfunction occurs in BD, our results provide evidence, not only for the value of research based in developmental systems neuroscience, but also for the importance of acquiring behavioral measures while subjects are being scanned and for using paradigms that yield patient-control differences in behavior. Routinely acquiring behavioral measures in clinical fMRI studies, as is done in cognitive neuroscience studies on control subjects, will improve investigators' ability to interpret neuroimaging data.

We expected to find between-group differences in behavior because, in a previous study (22), we found that children with BD demonstrate aberrant behavior and neurophysiology in emotional, but not non-emotional, contexts. Clinical researchers have debated the advantages and disadvantages of using fMRI paradigms that yield differences in behavior between patients and controls (48, 50). We suggest that paradigms showing such between-group differences in behavior may yield particularly informative neuroimaging results because, to the extent that these differences are relevant to patients' symptomatology, identifying group differences in neural activation while subjects perform the task may help to elucidate the pathophysiology of the illness (48). Of course it is important to ensure that such differences do not confound the interpretation of fMRI data. Here, we used an event-related design and appropriate statistical corrections to control for behavioral differences on a trial-by-trial basis.

We found that, when subjects' attention was directed to emotional aspects of neutral faces, the faces elicited more negative attributions in BD patients than in controls, as well as more limbic activation. Moreover, we found that the magnitude of this negative attribution predicted the degree of amygdala hyperactivation in patients, but not in healthy subjects. Thus, children with BD may have a tendency to perceive neutral stimuli more negatively than do healthy children. This behavioral deficit may result from limbic hyperactivation and contribute to clinical manifestations of pediatric BD. However, since this is an associational study, it is unclear if BD results from, or causes, the differences we observed in subjective ratings, RT, and limbic hyperactivation.

Neural hyperactivation in patients, compared to controls, was most evident bilaterally in the amygdala, and was also seen in the bilateral accumbens, putamen, and

left VPFC during emotional tasks (ratings of hostility or fearfulness). In particular, our data provide strong evidence implicating the left amygdala in dysfunctional face processing in pediatric BD, since between-group comparisons, within-group analyses, and correlations between BOLD signal and behavioral data all found an association between increased left amygdala activity and negative perceptions of neutral faces in the patients. Our finding of exaggerated amygdala response to faces in bipolar children is consistent with prior work in bipolar adults (51, 52). Furthermore, studies in controls indicate that fluctuating attentional task demands modulate amygdala activation while subjects process emotional stimuli (23, 53). Our results indicate that amygdala hyperactivation in pediatric BD may result from dysregulation of this emotion-attention mechanism, since patients engage the amygdala more than do controls only when attention is directed to the emotional components of a neutral stimulus.

There is now increasing evidence that children with BD have deficient social skills (54) and difficulty interpreting facial expressions (21). Here, we found that children with BD also misinterpret *neutral* facial expressions as being significantly more hostile and fear-inducing than do controls. Competence in social interactions requires proficient face processing and the over-identification of anger in neutral facial displays is associated with affective aggression and irritability (55). Since the latter behaviors are common in pediatric BD, further study of face processing in these patients may help elucidate the causes of functional impairment and suggest potential targets for therapeutic interventions.

A primary limitation of the current study is that, since most of our patients were medicated, it is unclear the extent to which our results are associated with BD itself or,

instead, reflect the patients' use of medication. The literature addressing the impact of medication on fMRI results in pediatric BD is limited, since 3 of 4 fMRI and 16 of 17 structural MRI studies in children with BD have included medicated patients (56-58). In adults with BD, one study did find frontal hyperactivation to be associated with medication; compared to unmedicated patients, medicated patients had increased dorsolateral PFC and anterior cingulate activation during a Stroop task (59). In contrast, two fMRI comparisons of medicated and unmedicated bipolar adults (60, 61), and one unpublished study in children with BD (62) found more marked differences in activation between unmedicated bipolar patients and controls than between medicated bipolar patients and controls, suggesting that medication may lead to Type II, rather than Type I, errors. In addition, previous work in adults with BD (51) or depression (63) indicates that lithium, antipsychotics and antidepressants may normalize neural activation in response to facial expressions. Due to ethical limitations of medication discontinuation, it is very difficult to obtain data in a sizable sample of unmedicated children with BD. Alternative designs may be needed to fully evaluate the role of neural circuitry dysfunction vs. that of medication. For example, a longitudinal study of youth at risk for BD may generate data in medication-free youth, document amygdala-based deficits similar to those found here, and relate them to future clinical manifestations of BD.

Another limitation is the high rate of comorbidity in our patients, although this rate is typical of that seen in other clinical samples of children with BD (64). When we compared patients with and without comorbid anxiety or ADHD, we did not find between-group differences in neural activation. However, the small sample sizes limit the interpretation of these negative results. Future research could determine the extent to

which patterns of neural activation in children with BD differ based on the presence of comorbid conditions. Further, given that hyperactivation of the amygdala, along with an attentional bias to emotional stimuli, has been demonstrated in other childhood mood disorders (23, 65-67), it will be important to determine whether the current results are specific to pediatric BD, as opposed to other childhood psychiatric illnesses. As previously noted, we anticipate that different psychopathologies would be associated with dysfunction in different neural circuits.

Finally, while half of our patients were euthymic, the other half presented in a depressed or hypomanic state during testing. When we compared euthymic vs. non-euthymic patients, we did not find between-group differences in neural activation. Again, caution should be used when interpreting these negative results due to the small sample sizes. Replication of our current results with a larger sample size will help to elucidate the impact of medication, comorbidity, and mood on the behavioral and neurological responses to faces in children with BD.

In sum, our results demonstrate abnormal emotion-attention interactions in pediatric BD that are associated with increased activation in the amygdala and ventral striatum and negative attributions of neutral faces. These findings add to prior studies of adults with BD. Taken together, the current and prior data support the view of serious, chronic, adult mental disorders as the end result of developmental perturbations in specific neural circuits.

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FIGURE LEGENDS

Figure 1: Greater neural activation in children with bipolar disorder (N=22) vs. controls (N=21) when rating their fear of neutral faces

Figure displays voxels where patients exhibited greater activation than controls on the afraid vs. nose width contrast for neutral faces. For the purpose of visual presentation, the threshold in this figure is set at $p<.01$ uncorrected. Peak voxels are in the left amygdala (x=-14, y=-2, z=-10), left accumbens (x=-10, y=4, z=-12), and right accumbens (x=12, y=4, z=-10).

Figure 2: Greater neural activation in children with bipolar disorder (N=22) vs. controls (N=21) when rating the hostility of neutral faces

Figure displays voxels where patients exhibited greater activation than controls on the hostility vs. nose width contrast for neutral faces. For the purpose of visual presentation, the threshold in this figure is set at $p<.01$ uncorrected. Peak voxels are in the left amygdala (x=-22, y=4, z=-18), left accumbens (x=-14, y=12, z=-10), left putamen (x=-24, y=8, z=-10), and left VPFC (x=-32, y=20, z=-16).

Supporting Text, Figure 3: Correlation between peak left amygdala activation on the hostile vs. nose width contrast and ratings of the hostility of neutral faces

Figure displays, in control and BD subjects viewing neutral faces, the correlation between peak left amygdala activation (x=-22, y=4, z=-18) on the hostility

vs. nose width contrast and hostility ratings. Correlation was significant in the BD sample ($r=.51$, $p=.014$), but nonsignificant in controls ($r=-.19$, $p=.40$).