Published in final edited form as:

J Am Acad Child Adolesc Psychiatry. 2012 March ; 51(3): 294–303. doi:10.1016/j.jaac.2011.12.008.

Amygdala hyperactivation during face emotion processing in unaffected youth at risk for bipolar disorder

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Disclosure: Ms. Olsavsky, Drs. Brotman, Deveney, Fromm, Towbin, Pine, and Leibenluft, and Ms. Rutenberg, and Mr. Muhrer report no biomedical financial interests or potential conflicts of interest.

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Abstract

Objective—Youth at familial risk for bipolar disorder (BD) show deficits in face emotion processing, but the neural correlates of these deficits have not been examined. This preliminary study tests the hypothesis that, relative to healthy comparisons (HC), both BD subjects and youth at-risk for BD (i.e., those with a first-degree BD relative) will demonstrate amygdala hyperactivation when viewing fearful and happy faces. The at-risk youth were unaffected, in that they had no history of mood disorder.

Methods—Amygdala activity was examined in 101 unrelated participants, 8-18 years old. Age, gender, and IQ-matched groups included BD (N=32), unaffected at-risk (N=13), and HC (N=56). During fMRI scanning, participants attended to emotional and non-emotional aspects of fearful and happy faces.

Results—While rating their fear of fearful faces, both BD and unaffected at-risk subjects exhibited amygdala hyperactivity vs. HC. There were no between-group differences in amygdala activity in response to happy faces. Post-hoc comparisons revealed that, in at-risk youth, familial risk status (offspring vs. sibling), presence of Axis I diagnosis (N=1 ADHD, 1 social phobia), and history of medication exposure (N=1) did not influence imaging findings.

Conclusions—We found amygdala hyperactivation in both unaffected at-risk and BD youth while rating their fear of fearful faces. These pilot data suggest that both face emotion labeling deficits and amygdala hyperactivity during face processing should receive further study as potential BD endophenotypes. Longitudinal studies should test whether amygdala hyperactivity to fearful faces predicts conversion to BD in at-risk youth.

Keywords

bipolar; anxiety; endophenotype; fMRI; faces

INTRODUCTION

Given the complex genetic architecture of bipolar disorder (BD), there is interest in identifying endophenotypes that might facilitate pathophysiologic discovery and the development of preventive interventions.¹⁻³ Like youth with BD, those at familial risk for the illness appear to have deficits in face emotion identification,^{4,5} making such behavioral deficits a candidate endophenotype. Specifically, on two face emotion identification tasks, both youth with BD and unaffected youth with a first-degree BD relative demonstrated deficits relative to healthy comparisons.^{4,5} However, to date, no published studies have examined neural correlates of face emotion processing in unaffected youth at risk for BD.

While prior studies have not examined neural activity in at-risk youth during face emotion processing, Surguladze et al. (2010) compared neural activity in adults with BD, their unaffected adult relatives, and healthy low-risk adults while subjects performed a gender identification task on fearful and happy faces.⁶ Compared to low-risk comparisons, BD probands and BD relatives showed hyperactivity in the amygdala in response to happy faces,

and in medial prefrontal cortex in response to happy and fearful faces. The relatives' mean age was 43 ± 13.8 years, beyond the age of risk for BD. Findings in younger at-risk subjects might indicate whether the degree of amygdala hyperactivity might ultimately be used to predict conversion to BD.

Here, we focused on unaffected youth at risk for BD, i.e., those without a history of a mood disorder. We excluded youth with a history of a mood disorder because an episode of depressive disorder may be an early presentation of BD. We included at-risk subjects with anxiety diagnoses or ADHD to avoid recruiting an unusually resilient sample. We focused specifically on the amygdala response to happy and fearful faces. We focused on happy faces because Surguladze et al. (2010) demonstrated amygdala hyperactivation in unaffected first-degree relatives of patients with BD in response to happy faces.⁶ We examined fearful faces because amygdala hyperactivation to fearful faces has been seen in both pediatric and adult BD populations.^{7,8} In addition, in a study using the same task as here, Beesdo et al. (2009) reported that youth with anxiety disorders had amygdala hyperactivation when viewing fearful faces and rating their own fear.⁹ This is consistent with other studies,⁹⁻¹² and is relevant because longitudinal community-based studies demonstrate an association between pediatric anxiety and adult BD,^{13,14} with one study showing a 5-fold increase in risk for adult BD in children with anxiety disorders.¹³

Given prior work demonstrating 1) longitudinal associations between pediatric anxiety and adult BD;^{13,14} 2) amygdala hyperactivity in response to fearful faces in patients with anxiety disorders or BD;¹⁰⁻¹² and 3) amygdala hyperactivity in response to happy faces in adults with, or at risk for, BD,⁶ we hypothesized that both unaffected youth at risk for BD and BD probands would demonstrate greater amygdala activity than healthy comparisons when viewing fearful or happy faces. Specifically, as noted above, Beesdo et al. (2009) found amygdala hyperactivity in youth with anxiety disorders while subjects rated their subjective fear of fearful faces (vs. passive viewing) using the same task as in this study.⁹ Therefore, we used this same contrast (subjective fear vs. passive viewing of fearful faces) as our main contrast of interest. Given the findings of Surguladze et al.⁶ in adults with a BD first-degree relative, and of Phillips et al. in adult probands with BD,^{15,16} we also examined this contrast while subjects viewed happy faces. Finally, in addition to our region of interest analysis focused on the amygdala, we performed an exploratory whole-brain analysis on these same contrasts.

METHOD

Subjects

Participants—101 unrelated youth (ages 8-18 years) were included. The three groups included age, gender, and IQ-matched youth, with 32 BD, 13 unaffected at-risk, and 56 HC. The study took place at the National Institute of Mental Health (NIMH), and was approved by the NIMH Institutional Review Board. Parental informed consent and child assent was obtained for all participants. Participants were recruited via advertisements in parenting magazines and on support groups' web sites, and distributed to psychiatrists nationally. From among these 101 subjects, data from 76 (28 BD, 48 HC) have been published previously. The at-risk data ($N=13$), which is the focus of this study, have not been published previously. Specifically, data from BD and HC subjects were used previously as follows: 28 BD and 24 HC in ¹⁷, 31 HC in ⁹, 31 HC in ¹⁸, and 20 BD and 29 HC in ¹⁹.

Inclusion Criteria—Clinical assessments were performed by Master's/Doctoral level clinicians trained to reliability ($\kappa>0.9$). Youth were evaluated using the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL). BD youth met criteria for “narrow-phenotype” bipolar disorder, i.e.,

history of at least one DSM-IV full duration hypomanic/manic episode with euphoric mood.²⁰ At-risk youth had at least one first-degree relative (parent and/or sibling) with BD confirmed by K-SADS-PL for child siblings with BD or, for parents or adult siblings with BD, the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCID-I/P)²¹ or the Diagnostic Interview for Genetic Studies (DIGS).²² To avoid recruiting an extremely resilient at-risk group, at-risk youth with ADHD or an anxiety disorder were included, although a history of major depressive episode or other mood disorder was exclusionary. HC had no personal history of psychiatric illness, and no first-degree relative with a mood or anxiety disorder.

Exclusion Criteria—For all participants, IQ<70 (determined by the Wechsler Abbreviated Scale of Intelligence²³), history of head trauma, neurological disorder, pervasive developmental disorder, medical illness preventing study participation, or substance abuse within two months were exclusionary. As noted above, mood disorder was also exclusionary in the at-risk group. Neither at-risk nor HC were on any psychotropic medications at the time of scanning.

Symptom Assessment—Within 48 hours of scanning, the Children's Depression Rating Scale (CDRS),²⁴ Young Mania Rating Scale (YMRS),²⁵ and Pediatric Anxiety Rating Scale (PARS)²⁶ were administered to at-risk and BD to assess symptoms of depression, mania, and anxiety.

Imaging Paradigm

Stimuli included gray scale photographs of 32 actors from several sets of previously-validated stimuli, including those of Ekman and Friesen,²⁷ Gur (www.uphs.upenn.edu/bbl/pubs/downloads/nptasks.shtml), and Tottenham and Nelson (www.macbrain.org/faces/index.htm). The paradigm used a rapid event-related design including 160 stimuli presented in 16 blocks over 14.2 minutes. Each block contained 10 randomly-ordered stimuli: two fixation crosses and two each of angry, happy, neutral, and fearful faces. The 16 blocks included 4 block types presented in random order and differentiated by task instructions: (1) "How hostile is the face?" (2) "How afraid are you of this face?" (3) "How wide is the nose?" (4) Passive viewing. "Hostility" was defined for younger participants. Instructions were presented at the beginning of each block for 3000 ms, and all stimuli were presented for 4000 ms. Each trial was followed by a variable inter-trial interval (750-1250 ms). Of note, this design relies on two distinct timing features. The first feature involves a 750-1250 ms inter-trial interval between events. This small degree of jitter is not designed to facilitate analysis of the fMRI data, but rather, maximizes task compliance by preventing expectant behavioral responses. The second feature involves the inclusion of randomly interspersed "null" trials, which allows deconvolution of responses to individual face-viewing events. Thus, as in Beesdo et al. (2009), to allow for accurate BOLD signal estimates of individual face-viewing trials, we insert two null trials (fixations) of the same duration as face-viewing events, appearing randomly within each eight-trial block.⁹ For this analysis, we selected two contrasts *a priori*, focusing on Afraid Ratings vs. Passive Viewing of fearful and happy faces.

fMRI Data Acquisition

Participants were trained on the task before scanning. Stimuli were displayed using Avotec Silent Vision Glasses (Stuart, FL). Functional MRI images were acquired on a General Electric Signa 3T magnet (GE Medical Systems, Waukesha, WI). Whole-brain functional T2*-weighted images were acquired using an echo-planar single-shot gradient positioned parallel to the plane including the anterior and posterior commissure [TR = 2000 ms, TE = 40 ms, 64 × 64 matrix, field of view 240 mm, 3.75 × 3.75 × 5 mm voxel size, 23 slices, 5

mm slice thickness]. High-resolution structural T1-weighted images were acquired [TR = 11.4 ms, TE = 4.4 ms, 256 × 256 matrix, field of view 256 mm, 180 slices, 1 mm slice thickness].

Behavioral Data Analysis

Behavioral variables included reaction time (RT) and afraid rating for each face type (fearful, happy). An 80% response rate cut-off was employed to ensure attention to the task. A chi-square test found no between-group difference in the proportion of participants excluded for poor response rate.

fMRI Data Analysis

Scans were attempted for 81 HC, 29 at-risk, and 53 BD. Data were excluded for low response rate (6 HC, 7 at-risk, 4 BD – chi-square=1.61, $p=0.45$), excessive motion (>3.5 mm motion in any direction; 10 HC, 6 at-risk, 10 BD – chi-square=1.61, $p=0.45$), significant image artifacts (2 HC, 3 BD – chi-square=2.21, $p=0.33$), and subject relatedness (7 HC, 3 at-risk, 4 BD – chi-square=0.19, $p=0.91$). Thus, number of scans excluded, and reason for scan exclusion, did not differ by group.

Image preprocessing was conducted in Statistical Parametric Mapping 8 (SPM8, Wellcome Trust Centre for Neuroimaging, London). After slice timing and motion correction, the data were spatially normalized to standard Montreal Neurological Institute (MNI) brain space, and voxel size was resampled to 2 × 2 × 2 mm. We employed an 8 mm Gaussian smoothing kernel followed by intensity normalization to ensure results could be interpreted as local percent signal change. Subject-level statistical models and appropriate contrasts were constructed in SPM8.

We employed a hypothesis-driven anatomical region of interest (ROI) analysis of the amygdala using the Afraid vs. Passive Viewing contrast for fearful and happy faces. This analytic technique is a standard SPM 8 analysis procedure. These analyses utilized previously validated bilateral anatomical amygdala masks.⁹ There were two steps to this analysis, one at the individual level and one at the group level. First, at the individual level, we extracted an average activation across the entire anatomically-defined amygdala for each participant for each contrast and face emotion. This resulted in 4 separate values for each participant (Afraid Rating vs. Passive Viewing of fearful faces and Afraid Rating vs. Passive Viewing of happy faces for the left amygdala, and parallel values for the right amygdala). Because we compared average activation across the entire amygdala, rather than multiple voxels or clusters within the amygdala, there were no multiple comparisons requiring correction.

Next, for group-level analyses, we submitted these 4 amygdala values per participant to one of four separate univariate analyses of variance (ANOVAs) conducted in SPSS. There were no covariates in these models. Each ANOVA examined group differences (BD, at-risk, HC) in the Afraid vs. Passive Viewing contrast, for a specific emotion (fearful or happy) and side (left or right). This analysis is equivalent to testing the interaction of group (BD, at-risk, HC) and attention state (Afraid Ratings, Passive Viewing).

When an analysis was significant, the interaction represented by the primary contrast was further decomposed to determine what was driving the between-group finding: activation while rating subjective fear or while viewing the faces. These *post hoc* analyses involved, again, calculating an average across the entire amygdala, this time for (1) Afraid Rating vs. Fixation and (2) Passive Viewing vs. Fixation, for the face emotion (i.e., fearful or happy) on which there had been between-group differences in the primary contrasts. Separate values were extracted for each amygdala side (i.e., left, right). As in the primary contrasts, these

analyses utilized previously validated bilateral anatomical amygdala masks.⁹ Also as in the primary contrasts, these average activation values were submitted to a univariate ANOVA in SPSS with group (BD, at-risk, HC) as the between subjects factor. Separate ANOVAs were calculated for each secondary contrast (i.e., Afraid Rating vs. Fixation, Passive Viewing vs. Fixation).

We also conducted an exploratory whole brain analysis for the two contrasts (i.e., Afraid Ratings vs. Passive Viewing of fearful faces; Afraid Ratings vs. Passive Viewing of happy faces). Unlike the primary analysis, where group contrasts were performed in SPSS, both the individual models and group contrasts were performed in SPM8 for this secondary, exploratory analysis. Consistent with the literature,²⁸ we used a statistical threshold of $p < 0.005$ and a voxel-wise extent threshold of $k = 20$ to identify clusters in which there were significant between-group differences in activation. As argued based on the computations of Lieberman and Cunningham (2009),²⁸ this threshold attempts to balance Type I and Type II errors in a fashion consistent with prior research in the behavioral sciences. Following group contrasts in SPM8, foci that differed significantly between groups were examined in a third step. To perform these analyses, percent signal change data were extracted from these defined clusters and assessed for group effects in a univariate ANOVA with accompanying *post hoc* t-tests in SPSS. When significant, the interaction represented by the primary contrast was decomposed to determine what was driving the between-group finding: activation while rating subjective fear or while viewing the faces.

Post Hoc Analyses for Medication, other Axis I disorders, and Mood State Effects

In at-risk subjects, we performed *post hoc* analyses to assess effects of family history of illness (i.e., affected sibling vs. affected parent), presence of Axis I diagnosis, history of medication use, and mood ratings. In BD, we performed *post hoc* analyses to assess effects of comorbid illnesses, mood state, and medications. Specifically, where we obtained significant findings in the primary analyses, we used t-tests to conduct the following *post hoc* analyses in at-risk youth: (1) at-risk subjects with BD siblings vs. at-risk offspring of BD parents; (2) at-risk with no Axis I disorder vs. HC; (3) medication-naïve at-risk vs. HC. In BD, we conducted the following *post hoc* analyses: (1) BD without comorbid illnesses vs. HC; (2) euthymic BD vs. HC; (3) correlation between number of medications in BD and activation; and (4) medicated BD vs. non-medicated BD. We also used Pearson correlations to examine associations between amygdala activity and behavioral performance, mood and anxiety ratings (BD and at-risk only), and number of medications (BD only).

RESULTS

Demographics and Clinical Features

Groups did not differ in age, IQ, or gender (Table 1). As expected, all at-risk were euthymic, confirmed via mood state ratings (data missing for one). Two at-risk had a psychiatric diagnosis (1 ADHD, 1 social phobia). Of note, only one at-risk had a lifetime history of an anxiety diagnosis. All but one at-risk youth were medication-naïve; the subject who was not medication-naïve was being treated with a short-acting stimulant which was withdrawn 48 hours before scanning.

When scanned, 59% of BD participants were euthymic (Table 1). Most BD patients (78%) had a comorbid diagnosis, most commonly an anxiety disorder (47%), and 75% were on at least one psychotropic medication.

Behavioral Data

Afraid Ratings of Fearful Faces—There were no between-group differences in subjective fear ratings elicited by fearful faces ($p=0.11$). There was a trend-level effect of RT ($p=0.07$), driven by at-risk being slower than HC ($p=0.02$) and BD ($p=0.05$). HC and BD did not differ.

Afraid Ratings of Happy Faces—Groups differed on subjective fear ratings of happy faces ($F_{2,98}=3.74$; $p=0.03$), with BD rating happy faces as more frightening than HC ($p=0.008$, $d=0.52$). At-risk did not differ from HC or BD (p 's >0.26); however the effect size of the at-risk vs. HC comparison was similar ($d=0.49$) to the BD vs. HC difference. There was a trend-level effect of RT ($F_{2,98}=2.96$; $p=0.06$), driven by BD taking longer than HC to rate subjective fear ($p=0.02$). At-risk did not differ from HC or BD.

Imaging Data

ROI ANALYSES

Fearful faces: Afraid Rating vs. Passive Viewing: Groups differed on right amygdala activity [$F_{2,98}=4.31$, $p=0.02$], with both BD ($p=0.05$) and at-risk ($p=0.01$) youth exhibiting amygdala hyperactivity relative to HC. BD and at-risk did not differ ($p>0.29$). The groups did not differ in left amygdala activation ($p>0.24$) (Figure 1).

To understand the between-group difference on Afraid Fearful vs. Passive Fearful, we compared activation during each condition to activation during fixation trials, utilizing the same averaged anatomical amygdala ROI activation used in the main contrast. On the Afraid Fearful vs. Fixation contrast, there was a trend in the right amygdala ($F_{2,98}=2.87$, $p=0.06$), primarily driven by BD hyperactivation compared to HC ($p=0.03$). BD did not differ from at-risk ($p=0.91$); at-risk did not differ from HC ($p=0.11$). There were no between-group differences in amygdala activation when comparing trials with passive viewing of fearful faces to fixation (all p 's >0.14).

Happy faces: Afraid Rating vs. Passive Viewing: There were no between-group differences in left ($F_{2,98}=0.50$, $p=0.61$) or right amygdala ($F_{2,98}=0.43$, $p=0.62$).

Post Hoc Analyses of Fearful faces: Afraid Rating vs. Passive Viewing – ROI Analysis

Familial Risk Status: There was no difference in right amygdala activity between siblings vs. offspring of BD probands ($p=0.16$). Furthermore, when comparing at-risk siblings ($N=5$) to HC ($p=0.02$, $d=0.88$) the difference was significant. Comparing at-risk offspring ($N=8$) to HC ($p=0.13$, $d=0.65$), the result was no longer significant, but there was a medium effect size.

Axis I Diagnoses/Comorbidity: The difference in amygdala activity between at-risk ($N=11$) and HC ($N=56$) remained significant when the two at-risk youth with Axis I diagnoses were removed from the analysis ($p=0.03$). When we compared BD without comorbid diagnoses ($N=10$) to HC ($N=56$), BD showed right amygdala hyperactivity ($p=0.02$).

Medications: When including only medication-naïve subjects at risk ($N=12$), at-risk showed amygdala hyperactivation compared to HC ($p=0.03$). Right amygdala activation did not differ between medicated BD ($N=24$) and medication-free BD ($N=8$) ($p=0.86$). Similarly, in BD, there was no correlation between number of medications and right amygdala activity ($p=0.93$).

Mood State: Right amygdala activity differed between euthymic BD ($N=19$) and HC ($N=56$) ($p=0.04$). YMRS and CDRS scores did not correlate with right amygdala activity in either BD or at-risk (all p 's >0.23). Right amygdala activity was not correlated with PARS in the at-risk group ($r=-0.20$; $p=0.55$), but exhibited a trend in the BD group ($r=-0.34$; $p=0.07$).

Behavioral Data: On the Afraid Fearful vs. Passive Fearful contrast, right amygdala activity did not correlate with either RT or ratings (p 's >0.15).

EXPLORATORY WHOLE BRAIN ANALYSES

Fearful Faces: Afraid Rating vs. Passive Viewing: Whole brain analysis ($p<0.005$ uncorrected, extent threshold $k\geq 20$) yielded a significant between-group difference only in a 78-voxel region with peak MNI coordinates of $[24 -26 -18]$ in the right parahippocampal gyrus/amygdala (Figure 2). This effect was driven by hyperactivation in BD and at-risk relative to HC ($p=0.02$ and $p<0.001$, respectively). In addition, at-risk showed hyperactivity ($p=0.05$) in this region compared with BD.

To understand this between-group difference, we compared activation during each condition to activation during fixation. On the Afraid Fearful vs. Fixation contrast, groups differ ($F_{2,98}=7.93$, $p=0.001$) in the same parahippocampal/amygdala area, driven by hyperactivation in BD vs. HC ($p=0.013$) and in at-risk vs. HC ($p<0.001$). There was a trend for hyperactivation in at-risk relative to BD ($p=0.09$). Passive Fearful vs. Fixation revealed no between-group difference ($F_{2,98}=1.76$, $p=0.18$).

Happy Faces: Afraid Rating vs. Passive Viewing: There were no suprathreshold voxels in the whole brain analysis.

DISCUSSION

It is important to examine potential BD endophenotypes in unaffected youth at familial risk because they are a critical population for understanding the developmental trajectory of BD, and because early interventions to prevent onset of the illness will be targeted toward this population. Face emotion labeling deficits have been demonstrated in both pediatric BD and at-risk populations,^{4,5,29} and thus are a candidate endophenotype for BD. Here, we built on these prior behavioral findings by comparing brain activity during face emotion processing in BD probands, unaffected at-risk youth, and healthy subjects. We hypothesized that unaffected at-risk youth and those with BD would exhibit amygdala hyperactivation while viewing fearful and happy faces. In this preliminary study, we found right amygdala hyperactivation in both BD and unaffected at-risk youth compared to HC while subjects rated their fear of fearful faces, with no difference between BD and at-risk. This finding remained significant even when controlling for at-risk familial status (i.e., offspring vs. sibling), and excluding the two at-risk subjects with Axis I diagnoses (one of whom was the only non-medication-naïve at-risk subject). In addition, co-morbid diagnoses and mood state did not account for our BD finding. In contrast to our finding with fearful faces, there was no between-group difference in amygdala activation to happy faces. We also performed an exploratory whole brain analysis, which supported the results of our ROI analysis. When considered in light of other research, our finding suggests that amygdala hyperactivation to fearful faces may be a candidate endophenotype in BD. Prior studies^{17,19} have examined Afraid Ratings vs. Nose Width ratings of neutral faces and have not consistently found amygdala hyperactivity in BD youth, suggesting that dysfunction on those conditions is not a candidate endophenotype.

An endophenotype is defined by five elements: (1) present in disease population; (2) present in population at familial risk; (3) state-independent; (4) heritable; and (5) co-segregates with

illness in the at-risk population.² Consistent with elements 1 and 2, we observed amygdala hyperactivation during processing of fearful faces in both at-risk and affected BD youth. Furthermore, amygdala hyperactivation was present in BD subjects when the analysis was limited to euthymic patients, consistent with element 3; since our at-risk sample was unaffected by mood disorders, they were by definition all euthymic. While data suggest that face emotion labeling performance is heritable,³⁰ the heritability of amygdala activity is unknown but worthy of study (element 4). Finally, demonstration of co-segregation (element 5) would require longitudinal studies to determine whether at-risk youth who demonstrate amygdala hyperactivation are at increased risk to develop BD, compared to at-risk youth who do not demonstrate such neural dysfunction.

Amygdala hyperactivation in response to fearful faces is potentially notable in BD and at-risk populations because such hyperactivation has also been found in youth with anxiety disorders,^{9,10} and two longitudinal community-based studies found that pediatric anxiety disorders predict BD in young adulthood.^{13,14} However, it is important to note that amygdala hyperactivation to fearful faces can be found in multiple mood and anxiety disorders, suggesting that amygdala hyperactivation may be a non-specific biomarker. By generating findings in at-risk youth, the current study might encourage future attempts to evaluate specificity definitively. Such attempts would require large, prospective studies that examine changes in amygdala function and clinical status over time among high and low-risk subjects with and without mood or anxiety symptoms.

The one prior fMRI study on face emotion processing in subjects at risk for BD included adults with a first-degree relative with BD.⁶ In that study, Surguladze et al. (2010) did not find amygdala hyperactivation in at-risk subjects during processing of fearful faces in the context of an implicit (gender identification) task, but did find such hyperactivation in response to happy faces. Here, we did not find between-group differences in amygdala activation during processing of happy faces. Our paradigm differed from that of Surguladze et al. (2010)⁶ i.e., explicit affect ratings here vs. implicit affect assessment/gender identification in the prior publication. Additionally, our finding in fearful, but not happy, faces may be developmentally-specific; perhaps fearful faces are more salient for at-risk youth than they are for adult BD relatives, while the opposite may hold for happy faces. Finally, given our small sample size, our failure to find a between-group difference in response to happy faces could represent a type II error.

Behaviorally, the groups in our study did not differ in the extent to which they found fearful faces frightening, although there was a trend for at-risk to have slower responses than HC and BD. Speculatively, the relatively slow responses of at-risk youth may reflect a compensatory reappraisal process, although in this small sample we did not find evidence for increased prefrontal engagement that might support such a hypothesis. BD rated happy face stimuli as more frightening than did HC; at-risk youth did not differ from either BD or HC, although the effect size of the at-risk vs. HC comparison was similar to the BD vs. HC comparison. This perception of happy faces as frightening could be interpreted as an emotional labeling deficit, as has been demonstrated in youth with BD and those at risk.^{4,5,31}

The whole brain analysis identified hyperactivation in at-risk and BD in a cluster that included voxels in both amygdala and parahippocampal gyrus (PHG), thus supporting our ROI analysis. Consistent with our ROI analysis, the happy faces whole-brain analysis revealed no between-group differences. PHG has been implicated in functional connectivity studies in BD. One study reported decreased connectivity with the amygdala in BD children and another reported increased connectivity with the cingulate in BD adults.^{31,32} One study reported increased PHG volume in at-risk children.³³ There are also a variety of volumetric

findings in the amygdala in the literature, with one study finding no difference in amygdala volumes in at-risk adolescents and adults,³⁴ and another meta-analysis finding increased amygdala volumes in bipolar adults, but decreased volumes in children with bipolar disorder.³⁵ In addition to the volumetric findings in amygdala, one recent study reported amygdala hyperactivation in at-risk adults compared to comparison adults while performing the Hayling sentence completion paradigm.³⁶

Post hoc analyses reveal that, relative to healthy subjects, BD youth showed amygdala hyperactivation when rating subjective fear of fearful faces vs. fixation. Youth at risk showed this pattern at a trend-level. This suggests that, in the case of the at-risk youth, the between-group difference in the primary analysis (Afraid Fearful vs. Passive Fearful) may be driven by contributions from each attention state (i.e., activation in at-risk greater than HC on afraid fear, activation in at-risk less than HC on passive fear), neither of which reaches statistical significance compared to fixation. Within BD, amygdala hyperactivation differed from controls when the sample was limited to euthymic BD and to those without comorbid Axis I diagnoses, suggesting that mood state and other comorbid diagnoses could not fully explain our BD findings. Another important distinction to make is that, although both BD and at-risk demonstrated amygdala hyperactivation, only BD (in addition to 2 at-risk youth) carried a psychiatric diagnosis at the time of scanning. Therefore, amygdala hyperactivation alone clearly does not produce the clinical symptoms of BD. Since amygdala hyperactivation is seen not only in BD, but in other psychiatric disorders, it is important to note that the utility of it as a potential biomarker is limited by its non-specificity.

There are several limitations to our study. Most importantly, the sample size of unaffected at-risk youth is small, since it is extremely difficult to recruit at-risk youth without a mood disorder who are willing to complete neuroimaging studies. Given the small at-risk sample, our findings should be considered preliminary, but are nonetheless important because they indicate that future studies of the circuitry mediating face emotion processing are warranted in at-risk youth, including possibly longitudinal studies. Our finding should also be considered preliminary because, while the task included several face emotions and attention states, the analysis included only a subset of these conditions. While our focus on fearful and happy faces was literature-driven,^{6,9} a model accounting for all conditions would be more robust statistically than our *a priori* selection of conditions and emotions. Lastly, while prior behavioral studies demonstrate face emotion labeling deficits in at-risk and BD subjects, categorical face emotion labeling was not assessed directly in this study.

Other limitations concern the nature of our BD and at-risk samples. While our inclusion of at-risk youth with anxiety disorders or ADHD may be considered a limitation, we did so because we did not want to select for a highly resilient at-risk population. Thus, our sample included two at-risk youth with Axis I diagnoses. Indeed, our primary finding remained when we removed these two youth from the sample. Second, there was heterogeneity of risk status in our sample, as some at-risk subjects had a BD sibling ($N=8$) while others had a BD parent ($N=5$). When we compared each subgroup to controls, the difference in amygdala activity remained significant (at least at trend-level, which might represent Type II error given the small subsamples). In general, heterogeneity in the at-risk sample, particularly in light of the small size, is also a significant limitation. Indeed, youth with affected first-degree relatives (either parent or sibling) were included in one group, and our study was not designed to tease apart effects of heterogeneity within the at-risk sample. Of note, however, only 15% ($N=2$) had an Axis I diagnosis, and the vast majority (92%, $N=12$) were medication-naïve.

In concert with prior data showing face emotion labeling deficits in youth at risk for BD, the current data suggest that amygdala activity during face emotion processing should receive further study as a potential endophenotype for BD. One component of this follow-up would include longitudinal imaging studies of at-risk youth to evaluate co-segregation of this endophenotype with BD. Additionally, elucidation of the connection between pediatric anxiety and BD in early adulthood is needed, and might assist with risk stratification in at-risk youth. Other relevant studies would include investigations of the heritability of amygdala hyperactivation during face emotion processing in healthy subjects as well as BD and their relatives. With further study, risk stratification and preventive interventions may become a reality in BD, mitigating the morbidity, mortality, and perhaps even incidence of the disorder.

Acknowledgments

This study was supported by the Intramural Research Program of the National Institute of Mental Health. Ms. Olsavsky's research was made possible through the Clinical Research Training Program, a public-private partnership supported jointly by the NIH and Pfizer Inc. (via a grant to the Foundation for NIH from Pfizer Inc.).

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Figure 1. Amygdala Region of Interest Analysis – Fearful Faces: Afraid Rating vs. Passive Viewing

Note: ROI = Region of Interest. ^aAt-risk vs. Healthy Comparisons $p=.01$; ^bBipolar Disorder vs. Healthy Comparisons $p=.05$

Figure 2. Whole Brain Analysis – Fearful Faces: Afraid Rating vs. Passive Viewing

Note: Maximal voxel of activation was found at Montreal Neurological Institute coordinates [24 -26 -18] with activation threshold $p < 0.005$ and $k = 78$.

^aAt-risk vs. Healthy Comparisons $p = .01$; ^bBipolar Disorder vs. Healthy Comparisons $p = .01$

Table 1

Demographics and Clinical Variables

	Bipolar Disorder	At Risk	Healthy Comparisons
Demographics			
N	32	13	56
Age in Years (SD)	14.7 (2.7)	14.0 (2.4)	14.0 (2.6)
Gender (% Male)	53%	54%	46%
Wechsler Scale of Intelligence FSIQ (SD)	111 (13)	113 (15)	112 (13)
Mood Ratings			
Young Mania Rating Scale Score (a)	9.6	3.7	—
Children's Depression Rating Scale Score (a)	28.3	19.7	—
Pediatric Anxiety Rating Score (a)	12.6	4.2	—
Current Diagnosis % (N)			
# of Diagnoses (SD)	2.7 (1.6)	1.0 (0.0)	—
Any Diagnosis	100% (32)	15% (2)	—
Bipolar Disorder	100% (32)	0% (0)	—
Any Anxiety Disorder	47% (15)	8% (1)	—
MDD	0% (0)	0% (0)	—
Psychosis	3% (1)	0% (0)	—
ADHD	41% (13)	8% (1)	—
ODD	22% (7)	0% (0)	—

Note: ADHD=Attention Deficit with Hyperactivity Disorder; FSIQ=Full scale IQ; MDD=Major Depressive Disorder; ODD=Oppositional Defiant Disorder.