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Subcortical volumetric correlates of anxiety in familial pediatric bipolar disorder: A preliminary investigation ☆

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ABSTRACT

Anxiety is a common comorbid condition in pediatric bipolar disorder (BD). However, there is little known about the effects of comorbidity on brain morphometry in this population. The aim of the present study was to examine subcortical correlates of anxiety in familial pediatric BD. The subject group comprised 120 children (mean age = 12 ± 3.3 years) with at least one parent diagnosed with BD. Bipolar offspring with BD were compared with bipolar offspring without BD on a measure of overall lifetime anxiety. A sub-sample of 20 bipolar offspring with BD (mean age = 14.6 ± 2.8 years) underwent magnetic resonance imaging (MRI) with a 3-T scanner. Correlational analyses were conducted between hippocampal and amygdalar volumes, and anxiety scores. The results showed significantly higher anxiety in bipolar offspring with BD compared to bipolar offspring without BD. There was a significant negative association between total hippocampal volume and anxiety scores. No significant association was found between total amygdalar volume and anxiety scores. Clinically, these findings suggest that anxiety comorbidity needs to be properly assessed and treated in the management of pediatric BD. This is the first study to show a negative association between hippocampal volume and anxiety in this population. The overlap between anxiety and familial pediatric BD suggests that anxiety may be one important area of future research in parsing out the heterogeneous nature and complex etiology of early-onset BD.

1. Introduction

Pediatric bipolar disorder (BD) is a chronic and highly comorbid psychiatric disorder associated with significant morbidity and mortality (Geller et al., 2000; Findling et al., 2001; Carter et al., 2003; Faedda et al., 2004). In recent years, magnetic resonance imaging (MRI) studies in pediatric populations with BD have begun to elucidate the pathophysiology of this lifelong illness in its earliest presentations. Despite the emerging emphasis on neuroanatomical circuits of mood regulation in adults and youth with BD (Mayberg, 1997; Lichter and Cummings, 2001; Blumberg et al., 2002; Chang et al., 2004), the structural brain abnormalities associated with early-onset BD remain largely unknown (Frazier et al., 2005a). In addition, a significant gap in knowledge exists regarding research on the effects of comorbidity on brain morphometry in youth with BD. Particularly, an emerging literature indicates the importance of associations between pediatric BD and comorbid anxiety disorders (Wozniak et al., 2002; Faedda et al., 2004; Dickstein et al., 2005a; Wagner, 2006). BD in youth is associated with increased risk for most anxiety disorders (Harpold et al., 2005). In one study, 77.4% of children and adolescents with BD had comorbid anxiety disorders (Dickstein et al., 2005a). Another study of adults with BD reported the highest rates of comorbid anxiety disorders (69.2%) in subjects with very early age at onset (<13 years) (Perlis et al., 2004). Studies show that earlier age at onset of BD in youth with comorbid anxiety disorders may indicate a more chronic and severe phenotype of BD (MacKinnon et al., 2003a,b; Perlis et al., 2004; Dickstein et al., 2005a). Moreover, researchers have speculated that anxiety in some children may be a prodrome of BD (Dickstein et al., 2005a) or that it may be a useful marker of risk for BD in at-risk populations (Henin et al., 2005).

The combination of BD and anxiety disorders poses a serious risk for children and adolescents as it increases the illness severity and contributes to additive symptoms. For instance, suicidal ideation and psychosis are more likely to occur when BD and panic disorder (PD) coexist in children and adolescents than in youth with PD only and

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non-BD psychiatric conditions (Birmaher et al., 2002). However, despite the critical role of anxiety in BD and the implications for clinical treatment, no published studies to date have examined neuroanatomical correlates of anxiety in early-onset BD.

A complicating factor in neuroimaging studies in BD is that structural MRI findings have not always been replicated. Reasons for these discrepancies include small sample sizes (and Type II error), differences between studies in image acquisition, processing and analysis, heterogeneity of subject samples due to severity of illness, genetic loading, age at onset, and exposure to psychotropic medication (Chang et al., 2006a). Comorbid psychiatric psychiatric conditions constitute another source of heterogeneity in subjects with BD. For example, adolescents with BD and comorbid attention-deficit/hyperactivity disorder (ADHD) show different striatal and prefrontal activation patterns compared with adolescents with BD without comorbid ADHD (Adler et al., 2005). Thus, including subjects with BD who do and do not have, for example, comorbid anxiety poses problems in "washing out" potential findings. Including correlations of brain morphometric findings with comorbid symptom severity may help tease out the interfering role that anxiety plays in such studies.

Nonetheless, neuroimaging studies have indicated the involvement of subcortical structures in the pathophysiology of the disorder (Strakowski et al., 2000). Thus, among the primary limbic regions for further study are the hippocampus and the amygdala, which have been implicated in neural circuits of mood regulation (Mayberg, 1997; Blumberg et al., 2002; Chang et al., 2004; Chang et al., 2005a; DelBello et al., 2006). These brain structures have also been implicated in neural circuits modulating anxiety behaviors (De Bellis et al., 2000, 2001; Bremner, 2002; Charney, 2003; Rauch et al., 2003). Thus, it is important to examine the effect of anxiety on these brain structures in youth with BD. This would contribute to a better understanding of the pathophysiology of BD, have implications for treatment and provide preliminary data for testing future hypotheses and models regarding the role of anxiety in pediatric BD.

Hippocampal volume has been found to be either decreased (Swayze et al., 1992) or unchanged (Altshuler et al., 1998; Hauser et al., 2000) in adults with BD. In addition, right hippocampal volume appeared decreased in monozygotic twins with BD compared with their discordant (healthy) twins (Noga et al., 2001). The findings in early-onset BD have also yielded inconsistent results. In a cohort of adolescents and adults with BD, a nonsignificant trend toward bilateral hippocampal volume reductions in adolescents with BD was reported (Blumberg et al., 2003) and two studies of youth with BD reported smaller hippocampal volumes than in controls (Frazier et al., 2005b; Bearden et al., 2008). On the other hand, two recent studies of pediatric BD did not find differences in hippocampal volume compared with controls (Chang et al., 2005a; Dickstein et al., 2005b). Volumetric neuroimaging studies in anxiety disorders have shown varying results, with reduced hippocampal volume in adults with post-traumatic stress disorder (PTSD) (Bremner et al., 1995; Bremner et al., 2003; Wignall et al., 2004) and no significant differences in hippocampal volume in traumatized populations (van Berkestijn and Kluiter, 1996; Bonne et al., 2001; Pederson et al., 2004). MRI studies of the hippocampus in childhood anxiety are sparse, have yielded varying results, and are limited to investigation of subjects with PTSD. Studies reported either no differences in hippocampal volume (Carrion et al., 2001), a trend towards larger left-hippocampal gray matter volume (De Bellis et al., 1999), or significantly larger hippocampus in children with PTSD compared with healthy controls (Tupler and De Bellis, 2006). As pointed out previously, it is important to note that the differences between studies are likely to be due to different methodologies, including different sample characteristics, image acquisition, and other related factors.

Volumetric amygdalar findings in adults with BD have been equivocal, with reports of similar (Swayze et al., 1992), decreased (Pearlson et al., 1997; Blumberg et al., 2003), or increased (Strakowski et al., 1999; Altshuler et al., 2000) amygdalar volumes. Amygdalar findings in pediatric BD are more consistent, as four studies of children and adolescents with BD found decreased amygdalar volumes (either bilaterally or unilaterally) in patients compared with healthy controls (Blumberg et al., 2003; DelBello et al., 2004; Chang et al., 2005a; Dickstein et al., 2005b). A fifth study with adolescents and young adults with BD reported a trend toward decreased left amygdalar volume in patients compared with controls and a positive correlation of age with amygdalar volume, a finding that was reversed in controls (Chen et al., 2004). In addition, a recent meta-analysis reported smaller amygdalar volumes in children and adolescents with BD than in controls (Preifer et al., 2008). Anxiety disorders in adults have been associated with amygdala volume reductions and hyper-responsiveness (Rauch et al., 2003). However, neuroimaging studies of childhood anxiety are limited and have yielded varying results, including increased amygdala volume in generalized anxiety disorder (GAD) (De Bellis et al., 2000), decreased left amygdalar gray matter volume in 17 youth with anxiety disorders of whom 13 were diagnosed with GAD (Milham et al., 2005), and unchanged amygdalar volume in youth with PTSD compared with controls (De Bellis et al., 2001). In addition, no significant correlations between anxiety and mood measures and amygdala volume reductions were observed (Milham et al., 2005).

Taken together, the aforementioned findings suggest that research examining neural correlates of anxiety in pediatric BD is warranted. Given that up to date no published studies have examined neural correlates of anxiety in early-onset BD, in the present study we attempted to investigate the associations between relevant subcortical brain regions and anxiety in this population. Children and adolescents with BD may be better suited for MRI studies than adults since early-onset disorders may be more familial and more severe (Schurhoff et al., 2000) and thus carry a higher likelihood of consistent biological abnormalities (Faraone et al., 2003). Furthermore, children often have had less exposure to psychotropic medications and less substance abuse, which may confound the interpretation of MRI data in adults. Because of an ongoing high-risk study, the present study sample consisted of offspring of parents with BD, thus enhancing the cohort for familial early-onset BD. Based on the literature, we hypothesized that among the offspring of parents with BD, children diagnosed with BD would have higher anxiety than children not diagnosed with BD. We also hypothesized that anxiety would be negatively correlated with hippocampal volume and amygdalar volume in offspring diagnosed with BD.

2. Methods

2.1. Subjects and assessment

This protocol was approved by the Stanford University Panel of Medical Research in Human Subjects. Subjects were recruited for an ongoing high-risk phenomenology study of bipolar offspring of parents with BD. Families were recruited from the Stanford Adult Bipolar Disorders Clinic, the Stanford Pediatric Bipolar Disorders Program, physician referrals, local adult bipolar support groups, and the surrounding community. A total of 120 subjects participated in this protocol, and a sub-sample of 20 bipolar offspring with BD participated in the neuroimaging protocol of this study.

After obtaining oral and written informed consent from parents and oral and written assent from their offspring, we conducted semistructured interviews. Participants had at least one biological parent with bipolar I or II disorder. Parents were diagnosed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al., 1995), and were interviewed for psychiatric history of first- and second-degree relatives following the Family History-Research Diagnostic Criteria (FH-RDC) (Andreasen et al., 1977). Bipolar offspring were assessed using the Affective Disorders Module of the Washington Schedule for Affective Disorders and Schizophrenia for School-Age Children (WASH-U-KSADS) (Geller et al., 1996, 2001). All evaluations were conducted by either a child and adolescent psychiatrist (KC) or a trained master's level research assistant with 3 years' experience in

psychiatric interviewing. Evaluators were aware of parental diagnosis. Inter-rater reliability was established by rating videotaped interviews, observing a trained rater, observing trained rater interviews, and performing interviews with observation by a trained rater, as outlined by Geller and colleagues (Geller et al., 1998). Both the parents and the children were interviewed and parents were euthymic at the time of their own and their child's interview. Diagnostic decisions were ultimately made by a board-certified child and adolescent psychiatrist (KC) based on personal interview, discussion with the research assistant and written notes of parental and offspring responses to interview questions. Current and lifetime diagnoses were established according to DSM-IV criteria (American Psychiatric Association, 1994).

Inclusion criteria for all bipolar offspring were age 6–18 years and a biological parent with bipolar I or II disorder. Inclusion criteria for bipolar offspring with BD participating in the MRI protocol were age 9–18 years, biological parent with bipolar I or II disorder, and diagnosis of bipolar I disorder by the WASH-U-KSADS. Exclusion criteria were presence of a pervasive development disorder (such as autism or Asperger's disorder), a neurological condition (such as a seizure disorder), a substance use disorder, IQ less than 80, or presence of metallic implants or braces (for participants in the MRI portion of the study). Age at onset of BD was determined retrospectively as the earliest period to the closest month when patients met criteria for a manic or depressive episode, as defined by the DSM-IV.

The Child Behavior Checklist (CBCL; Achenbach and Edelbrock, 1983; Achenbach, 1991) and the Dimensions of Temperament Survey — Revised (DOTS-R; Windle and Lerner, 1986) were administered to all bipolar offspring. The CBCL was completed by one of the subject's parents, most frequently the mother. The clinical scales of the CBCL contain a Total Problem Scale, two broadband dimensions (Internalizing Problems and Externalizing Problems), and eight cross-informant syndromes (Aggressive Behavior, Delinquent Behavior, Somatic Complaints, Anxious/Depressed, Attention Problems, Social Problems, Thought and Withdrawal Problems). Past research with the CBCL has demonstrated its validity and reliability in clinical settings (Biederman et al., 1993; Bird et al., 1987). The DOTS-R was completed by the subject (if older than 12 years of age) or by one of the parents (if younger than 12 years of age). The DOTS-R is a 54-item instruments that measures nine temperament characteristics: Activity Level–General, Activity Level–Sleep, Flexibility–Rigidity, Approach–Withdrawal, Rhythmicity–Sleep, Rhythmicity–Eating, Rhythmicity– Daily Habits, Task Orientation, and Mood. This instrument has good to excellent psychometric properties (Windle and Lerner, 1986), and there is good concordance between parent and child ratings (Luby and Steiner, 1993).

Subjects with BD were all outpatients at the time of scanning. They were administered the clinician-rated Young Mania Rating Scale (YMRS) (Young, 1978; Fristad et al., 1995) and completed the Children's Depression Inventory (Kovacs, 1985), with the help of a parent if they were younger than 12 years of age. The IQ was assessed with the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). Stimulant medication was discontinued for 24 h before the scan, primarily due to a concurrent, separate functional MRI study of attention. Patients with BD were allowed to continue any other current medications such as mood stabilizers or antidepressants due to the risk of mood destabilization.

Demographic data collected included age, gender, parental occupation and level of education, household income, ethnic status, birth order and number of siblings, and parental and child age of illness onset. The socioeconomic status of the families was measured using the Four-Factor Index of Social Status (Hollingshead and Redlich, 1958).

2.2. Anxiety index

An anxiety index was created retrospectively and was intended to measure degree of anxiety symptoms rather than establish threshold for a specific anxiety disorder. Different anxiety symptoms from three assessment measures were grouped together. The rationale derives from recent theories postulating that all anxiety disorders have common neural underpinnings (Pine and Grun, 1999). The aim was to establish a measure of lifetime anxiety exposure. It was assumed that lifetime anxiety would have the greatest impact on brain morphometry. However, most anxiety instruments measure current symptoms only. Therefore, the anxiety index in this study incorporated a total of 32 questions from three assessment instruments. The index constituted a continuous measure and included all 14 questions from the anxiety section of the WASH-U-KSADS addressing PD, GAD, separation-anxiety disorder (SAD), social phobia (SP), and specific phobia (i.e., "unrealistic worry about future", "tension, unable to relax"). We did not include questions pertaining to PTSD and obsessive–compulsive disorder (OCD), as we wanted to capture more general symptoms of anxiety, and these diagnoses are typically excluded from treatment studies of anxiety in children. The index also included 14 CBCL items (i.e., "too fearful or anxious", "fears going to school") and four DOTS-R items (i.e., "it takes me no time at all to get used to new people") addressing symptoms of anxiety. Because of the greater validity of clinician-obtained ratings (Friman et al., 2000; Jewell et al., 2004), the questions from the diagnostic interview were weighted twice as much as the CBCL and DOTS-R questions. Thus, a maximum of 2 points were possible for each question on the WASH-U-KSADS, while a maximum of 1 point was possible on each question from the CBCL and DOTS-R. The highest possible score was 46.

2.3. Magnetic resonance imaging and image analysis

Magnetic resonance images of each subject's brain were acquired with a GE 3T scanner (GE Medical Systems, Milwaukee). Coronal images were acquired with a three-dimensional volumetric radiofrequency spoiled gradient echo with the following scan parameters: TR = 35 ms, TE = 6 ms, flip angle = 45°, number of excitations = 1, image matrix = 256 × 192 pixels, field of view = 24 cm, slice thickness = 1.5 mm, 124 slices, acquired resolution = $1.5 \times 0.9 \times 1.2$ mm^3. The images were reconstructed as a $124 \times 256 \times 256$ matrix with a $1.5 \times 0.9 \times 0.9$ mm^3 spatial resolution.

Image data were imported into the program BrainImage 5.29 (Stanford Psychiatry Neuroimaging Laboratory; http://spnl.stanford.edu) for semi-automated image processing and quantification. A detailed description of the overall method for image processing, measurement, and calculations for total brain volume (TBV), total

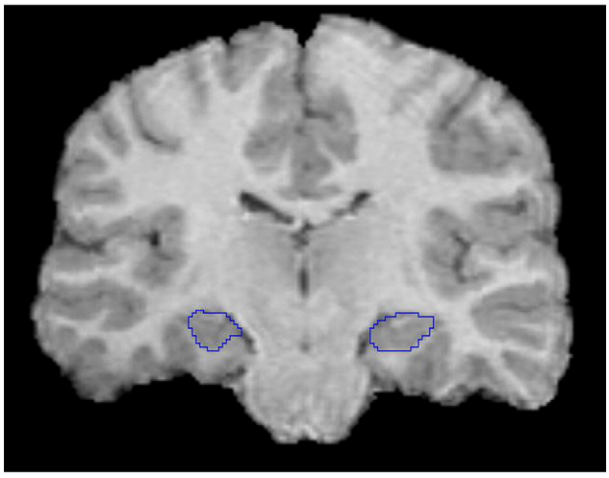

Fig. 1. Outline of the left and right hippocampi on the positionally normalized brain stack in coronal orientation. Hippocampi were traced starting at the slice where a clear distinction between amygdala and hippocampus was first visible and outlined proceeding posteriorly until the structure disappeared. The superior white matter tract extending from the temporal lobe was used as the inferior border of the hippocampus, the medial border was defined by CSF and the pons, where present, and the lateral border was marked by CSF or white matter tracts on the lateral edge of the hippocampus.

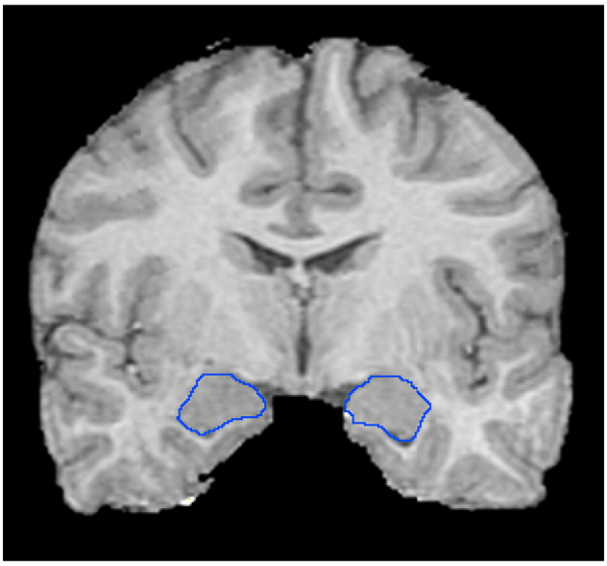

Fig. 2. Outline of the left and right amygdalae on the positionally normalized brain stack in coronal orientation. The most superior white matter tract extending from the temporal lobe marked the inferior border, CSF marked the medial border, the entorhinal sulcus marked the superior border, and a thick central white matter tract of the temporal lobe was used as the lateral border of the amygdala.

cerebral volume, and total brain tissue has been provided elsewhere (Chang et al., 2005a).

Subcortical regions were outlined manually by reliable raters (intra-class inter-rater reliability >0.9), on positionally normalized brain stacks in a coronal orientation perpendicular to the horizontal plane defined by the anterior and posterior commissures.

Hippocampi were traced starting at the slice where a clear distinction between amygdala and hippocampus was first visible and outlined proceeding posteriorly until the structure disappeared. The superior white matter tract extending from the temporal lobe was used as the inferior border of the hippocampus, the medial border was defined by CSF and the pons, where present, and the lateral border was marked by CSF or white matter tracts on the lateral edge of the hippocampus (Fig. 1).

Amygdalae were traced starting on the slice with the thickest anterior commissure and following the structure posteriorly until it disappeared. The most superior white matter tract extending from the temporal lobe marked the inferior border, CSF marked the medial border, and a thick central white matter tract of the temporal lobe was used as the lateral border of amygdala (Fig. 2).

The volume measurements of the subcortical structures were done on the positionally normalized segmented white, gray, and CSF stacks.

Table 1
Demographic characteristics of overall cohort of bipolar offspring.

	Bipolar offspring with BD	Bipolar offspring without BD
N	37	83
Mean Age, years (SD)	12.8 (3.3)	11.7 (3.3)
Gender, % male	76	54
SES (SD)	3.9 (0.9)	3.9 (0.9)
Race (%)		
African-American	1 (3)	3 (4)
Hispanic	2 (5)	3 (4)
Asian	0 (0)	8 (10)
Caucasian	34 (92)	68 (82)
Comorbid diagnoses (%)		
ADHD	32 (87)	36 (43)
Anxiety disorder	13 (35)	18 (22)
Oppositional defiant disorder	23 (62)	12 (15)
GAF	53 (9.6)	75 (12.2)

SD = standard deviation, SES = socioeconomic status, ADHD = attention-deficit/hyperactivity disorder.

Table 2
Demographic characteristics of subjects included in MRI protocol.

	Bipolar offspring with BD
N	20
Mean Age, years (SD)	14.6 (2.8)
Gender, % male	80
SES (SD)	4.2 (0.8)
Race (%)	
African-American	1 (5)
Hispanic	1 (5)
Asian	0 (0)
Caucasian	18 (90)
I.Q. mean (SD)	109.5 (11.4)
Handedness (% right)	95
Comorbid diagnoses (%)	
ADHD	17 (85)
Anxiety disorder	7 (35)
Oppositional defiant disorder	12 (60)
YMRS	15.4 (8.7)
CDI	15.3 (8.7)
GAF	54.3 (8.0)
Duration of illness, years (SD)	1.7 (1.8)
Past psychotropic medication	
Exposure (%)	
Stimulants	60
TCAs	15
SSRIs	65
Atypical ADs	50
Lithium	35
Valproate	45
Antipsychotics	35
Any mood stabilizer	65

SD = standard deviation, SES = socioeconomic status, ADHD = attention-deficit/hyperactivity disorder, YMRS = Young Mania Rating Scale, CDI = Children's Depression Inventory, TCAs = tricyclic antidepressants, SSRIs = selective serotonin reuptake inhibitors, Ads = antidepressants.

Raters who conducted morphometric analyses were blind to the diagnosis of each subject.

2.4. Statistical analyses

Data distributions were examined for normality in the total sample of 120 bipolar offspring with and without BD. The anxiety index data were not normally distributed and were therefore normalized using square root transformation before applying parametric tests. Analysis of variance (ANOVA) was used first, to compare bipolar offspring with and without anxiety disorders to test the validity of the scale and, second, to compare the two groups of bipolar offspring (bipolar offspring with BD and bipolar offspring without BD) on the anxiety scale. Also, analyses of covariance (ANCOVA) were performed when appropriate to test for the effects of gender and age. Cronbach's alpha coefficient was used to test the internal consistency of the anxiety scale. For those analyses, a P value of ≤ 0.05 was chosen as the significance threshold.

All volumetric data concerning the brain structures of interest were normally distributed in the sub-sample of 20 bipolar offspring participating in the MRI protocol of this study. Partial correlation coefficients, controlling for total brain volume (TBV), were calculated to examine the correlation between anxiety symptoms and the morphometric regions of interest (ROIs). Age and gender were examined, but did not yield significant associations with the variables of interest and were therefore not included as covariates. To adjust for multiple comparisons, the significance level was set at $P \leq 0.025$.

3. Results

3.1. Cohort

The mean age of the total cohort of 120 bipolar offspring was 12 ± 3.3 years; age range was 6.2–18.8 years. Eighty-three participants were bipolar offspring without BD (54% male) and 37 participants

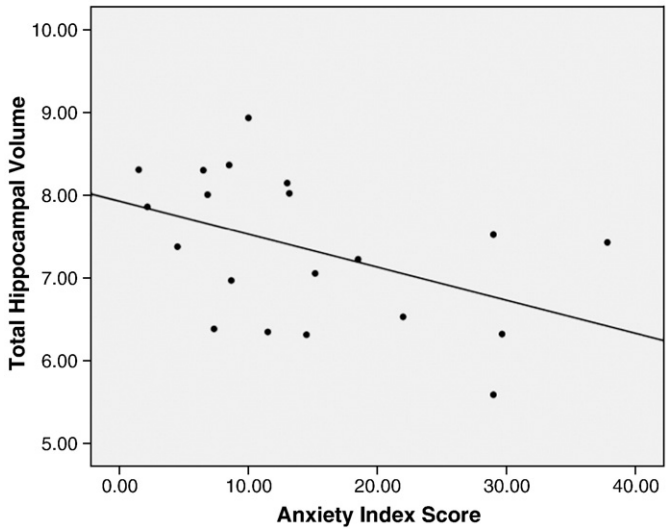

Fig. 3. Correlation between total hippocampal volume and anxiety index score in bipolar offspring with BD ($r=-0.46$, $P=0.025$).

were bipolar offspring with BD (76% male). Descriptive statistics of all bipolar offspring appear in Table 1. There were no significant age differences between the two groups of bipolar offspring ($F(1, 119)=2.99$, $P=0.09$), but there were gender differences ($\chi^2=4.95$, $P=0.03$), with more males in the group of bipolar offspring with BD.

A sub-sample of 20 bipolar offspring with BD participated in the MRI protocol of this study. This sub-sample has been described elsewhere (Chang et al., 2005b) and demographic variables are summarized in Table 2.

3.2. Anxiety index

Cronbach's alpha coefficient ($\alpha=0.82$) showed very good internal consistency of the anxiety index and demonstrated that the index's 32 items were associated with the same construct. In the total sample, the anxiety index differentiated well between bipolar offspring with and without anxiety disorder diagnoses ($F(1, 118)=57.85$, $P=0.000$) with children with anxiety disorder diagnosis showing higher anxiety scores 18.8 ± 5.9 than children without anxiety disorder diagnosis 8.6 ± 6.7. This result remained significant even after covarying for age differences between the two groups ($F(2, 117)=28.19$, $P=0.000$). The effect size of this finding was Cohen's $d=1.61$, indicating a large effect.

Consistent with the hypothesis, in the total sample of 120 bipolar offspring, ANOVA indicated mean differences in anxiety symptoms between bipolar offspring with BD and bipolar offspring without BD ($F(1, 118)=12.64$, $P=0.001$), with children with BD showing higher anxiety scores 14.9 ± 8.7 than children without BD 9.6 ± 6.3. The overall mean anxiety score was 11.2 ± 7.5. This result remained significant even after covarying for gender differences between the two groups ($F(2, 117)=6.27$, $P=0.003$). The effect size of this finding was Cohen's $d=0.68$, indicating a medium effect.

Table 3
Mean (SD) brain regional raw volumes (in cubic centimeters) in bipolar subjects.

Brain region	Bipolar
Total brain volume	1484.3 (128.6)
Total hippocampal volume	7.35 (0.90)
Left hippocampal volume	3.62 (0.55)
Right hippocampal volume	3.74 (0.42)
Total amygdalar volume	3.93 (0.40)
Left amygdalar volume	2.00 (0.31)
Right amygdalar volume	1.94 (0.20)

Table 4
Correlations between regions of interest and anxiety index score.

Brain Region	*r*	*P*
Total hippocampal volume	−0.46	0.025
Left hippocampal volume	−0.48	0.020
Right hippocampal volume	−0.36	0.066
Total amygdalar volume	−0.11	0.325
Left amygdalar volume	−0.06	0.411
Right amygdalar volume	−0.13	0.296

3.3. Hippocampal and amygdalar volumes

Consistent with the hypothesis, in the sub-sample of 20 bipolar offspring with BD, there was a significant negative correlation between anxiety symptom scores and total hippocampal volume ($r=-0.46$, $P=0.025$) (Fig. 3). Anxiety symptom scores were not correlated with total amygdalar volume ($r=-0.11$, $P=0.325$). We sought to determine post hoc and in exploratory bilateral analyses whether left or right volume in the hippocampus was contributing to this finding. There was a significant correlation between anxiety and left hippocampal volume ($r=-0.48$, $P=0.020$). There were no other significant correlations between anxiety and left and right ROIs.

The brain regional volumes for bipolar subjects and the correlations between anxiety symptoms and ROIs are presented in Tables 3 and 4, respectively.

4. Discussion

Consistent with the a priori hypotheses, two major findings emerged from this study. First, we found that in a high-risk group of children and adolescent offspring of parents with BD, offspring diagnosed with BD have significantly higher levels of lifetime anxiety compared with offspring of bipolar parents without a diagnosis of BD. Second, the finding of a negative association between hippocampal volume and anxiety is the first report of this finding in youth with BD.

The results of higher rates of anxiety in offspring diagnosed with BD compared with offspring without BD are consistent with the growing adult and pediatric literature indicating high rates of comorbidity between anxiety and BD (Masi et al., 2001; McElroy et al., 2001; Birmaher et al., 2002; Dickstein et al., 2005a; Harpold et al., 2005; Simon et al., 2003), as well as findings from studies examining anxiety in high-risk offspring (Grigoroiu-Serbanescu et al., 1989; Hammen et al., 1990; Henin et al., 2005). Familial BD has been associated with elevated risk for PD (Edmonds et al., 1998; Johnson et al., 2000; MacKinnon et al., 2002; MacKinnon et al., 2003a,b), SAD (DelBello and Geller, 2001), and phobias (Edmonds et al., 1998). PD is one of the most studied comorbid anxiety disorders in familial BD (MacKinnon et al., 2002; Wozniak et al., 2002; MacKinnon et al., 2003a,b). However, when studying pediatric anxiety, it is important to look outside of PD and examine overall anxiety symptoms and behaviors, because PD occurs less frequently in childhood.

From a clinical perspective, the findings suggest that anxiety symptoms and behaviors need to be properly assessed and treated in the management of pediatric BD, even if a full anxiety disorder is not present. This is important in light of findings that comorbid anxiety in BD might be an important risk factor for suicide (Young et al., 1993). Other investigators have demonstrated that the presence of anxiety comorbid with BD significantly increases the illness severity, contributing to higher rates of non-remission and more severe mood episodes over time (Feske et al., 2000; McElroy et al., 2001).

Because offspring with BD had higher levels of lifetime anxiety than offspring without BD, anxiety symptoms in such high-risk children may signify risk for eventual BD development (Dickstein et al., 2005a; Henin et al., 2005). Also, findings from a familial risk study suggest that the combination of BD and anxiety might be a "distinct clinical entity linked to very early onset" of BD (Wozniak et al., 2002). Thus, future studies examining the role of anxiety in early-onset BD have the potential of

clarifying neural mechanisms that may be linked to a specific clinical phenotype of BD. This objective is also critical to the long-term goal of preventative interventions in youth at high risk for the development of the illness (Chang et al., 2006b, 2007).

Regarding the finding of a significant negative association between hippocampal volume and anxiety in children and adolescents with BD, there are only a few studies examining the hippocampus in early-onset BD (Blumberg et al., 2003; Chang et al., 2005a; Dickstein et al., 2005b; Frazier et al., 2005b, 2008; Bearden et al., 2008), and none examining the relationship of anxiety symptoms and behaviors to hippocampal morphometry. This finding was driven by a negative correlation between anxiety and left hippocampal volume.

One possible interpretation of the negative association between anxiety symptom scores and hippocampal volume is a neural mechanism of action via high glucocorticoid levels. Overactivity of the HPA axis, and thus increased secretion of cortisol, a glucocorticoid and a steroid hormone released in response to stress, has been associated with pediatric PTSD and depression (Carrion et al., 2002). Furthermore, the children in the PTSD study had high comorbidity with other anxiety disorders such as SAD, specific phobia, and social phobia. The researchers did not find a difference in cortisol levels between children diagnosed with PTSD and children with subthreshold PTSD symptoms (Carrion et al., 2002). Therefore, high cortisol levels might not be specific to PTSD, but also to symptoms of anxiety such as nightmares and social avoidance. Because in the present study the anxiety index included measures of anxiety symptoms, one could speculate that cortisol levels may be positively associated with anxiety scores, thus mediating the relationship between anxiety and hippocampal volume. It is possible that increased anxiety means increased subjective experience of stress, leading to increased brain glucocorticoid levels. Since the hippocampus has a high concentration of glucocorticoid receptors, it is particularly susceptible to the effects of cortisol (De Kloet et al., 1998). Chronically high levels of cortisol in the brain can cause a reduction of hippocampal volume via neuronal cell death (Sapolsky et al., 1986; Bremner et al., 2000).

The results of the present study did not indicate significant associations between anxiety index scores and amygdalar volume. Interestingly, although this association was not significant, it was in the predicted direction. This finding suggests that in children already diagnosed with BD, anxiety does not affect overall amygdalar volume, yet confounding variables may have played a role in this finding. The ability to detect effects may have been limited by inadequate power. It is also possible that only some anxiety disorders may have an effect on the amygdala. The spectrum of anxiety symptoms measured by the anxiety index in the present study would not capture this effect. Another possible interpretation is that the relationship between the amygdala, which mediates emotional processing, and anxiety might not be manifested on the structural volumetric brain level but rather on the functional level. For instance, functional MRI studies indicate face- and emotion-processing deficits in pediatric BD compared with controls, with BD youth misinterpreting emotional facial expressions (McClure et al., 2005) and misinterpreting neutral faces as being significantly more hostile and threatening than do controls (Rich et al., 2006). Further, a recent study reported impaired functional connectivity between the amygdala and temporal association cortical regions critical to evaluation of emotional expressions and social stimuli in pediatric BD (Rich et al., 2008). Clearly, more research is needed to examine the relationship between anxiety and the amygdala in this population.

Overall, the findings of the present study should be viewed as preliminary because of several limitations. First, one major limitation is that the anxiety symptom measure was an index of overall anxiety symptoms and behaviors. The index was created retrospectively and thus could be subject to bias (as well, some of the items included in the anxiety index were based on retrospective parent report). The index did not reflect anxiety severity or duration of anxiety, since only lifetime anxiety was assessed in this cohort. Future studies should attempt to use well-established reliable and validated measures of lifetime anxiety and include specific comorbid anxiety disorders that could potentially have differential effects on brain morphometry in pediatric BD. Second, the findings might be unique to familial early-onset BD, limiting generalizability to other cohorts of children with BD. Third, it is important to consider that structural brain changes can be due to genetically mediated mechanisms (inborn), or to environmental causes. Amygdala–hippocampal complex volume appears to be genetically mediated in families with a dominant pattern of transmission (Lawrie et al., 2003). Future studies should address parental as well as offspring brain structures to help distinguish the causes of volumetric abnormalities. Fourth, it is important to recognize that 85% of 20 bipolar offspring with BD in this study had a diagnosis of ADHD, the effects of which on the findings are unknown. Although, previous research has shown that ADHD and anxiety disorders segregate independently in families (Biederman et al., 1992; Braaten et al., 2003), anxiety disorders have also been found to occur more in the presence of ADHD (Braaten et al., 2003). Fifth, the majority of our subjects were male. Although we did not find gender effects, recent studies underscore the importance of examining gender effects on brain morphometry in pediatric BD (Frazier et al., 2008). Given that puberty marks a normative surge in sex hormones paralleled by neuromaturational changes in the brain, future studies with sufficient numbers of subjects should examine gender effects in this population. Finally, we were not able to control for lifetime medication exposure, which could account for variability in brain morphometry. Medication effects on brain volume and anxiety levels are largely unclear. Research indicates that antidepressant treatment might have a neuroprotective effect on hippocampal neurons (Santarelli et al., 2003; Sheline et al., 2003). Further, evidence from preclinical studies suggests that the regulation of hippocampal plasticity might be associated with neuroprotective effects of mood stabilizers (Frey et al., 2007). Future studies should examine the relationship between antidepressant treatment as well as other pharmacologic agents and brain morphometry in this population. Children and adolescents with more anxiety symptoms might have increased exposure to antidepressants, which could have an effect on hippocampal volume. It is possible that exposure to specific medication treatments represents a potential mediating or moderating mechanism in the relationship between behavioral symptoms and brain structures.

Despite these limitations, the findings from this preliminary investigation contribute to a better understanding of the neuropathophysiology of familial pediatric BD and have important clinical and research implications. It is possible that longstanding anxiety may further impair hippocampal functioning, leading to further limbic dysfunction and mood cycling. Future research needs to investigate further the role of the hippocampus and other prefrontal–amygdalar areas in early-onset BD and to delineate potential neural mechanisms contributing to BD development. Longitudinal studies are especially needed to determine the developmental trajectory of structural abnormalities and the relationship between brain structures of interest and comorbid presenting symptoms such as anxiety in BD.

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