

Structural Brain Magnetic Resonance Imaging of Limbic and Thalamic Volumes in Pediatric Bipolar Disorder

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Background: Youths with bipolar disorder are ideal for studying illness pathophysiology given their early presentation, lack of extended treatment, and high genetic loading. Adult bipolar disorder MRI studies have focused increasingly on limbic structures and the thalamus because of their role in mood and cognition. On the basis of adult studies, the authors hypothesized a priori that youths with bipolar disorder would have amygdalar, hippocampal, and thalamic volume abnormalities.

Method: Forty-three youths 6–16 years of age with DSM-IV bipolar disorder (23 male, 20 female) and 20 healthy comparison subjects (12 male, eight female) similar in age and sex underwent structured and clinical interviews, neurological examination, and cognitive testing. Differences in limbic and thalamic brain volumes, on the logarithmic scale, were tested using a two-way (diagnosis and sex) univariate analysis

of variance, with total cerebral volume and age controlled.

Results: The subjects with bipolar disorder had smaller hippocampal volumes. Further analysis revealed that this effect was driven predominantly by the female bipolar disorder subjects. In addition, both male and female youths with bipolar disorder had significantly smaller cerebral volumes. No significant hemispheric effects were seen.

Conclusions: These findings support the hypothesis that the limbic system, in particular the hippocampus, may be involved in the pathophysiology of pediatric bipolar disorder. While this report may represent the largest MRI study of pediatric bipolar disorder to date, more work is needed to confirm these findings and to determine if they are unique to pediatric bipolar disorder.

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Bipolar disorder is one of the most severe neuropsychiatric disorders at any age and is among the most disabling of psychiatric conditions that affect youths (1, 2). Although the chronology of underlying structural brain abnormalities in this population is unknown, such abnormalities may represent disruptions in typical brain growth resulting from an interplay of genetic and environmental factors. MRI studies are critical for advancing our knowledge of brain regions involved in the pathophysiology of pediatric bipolar disorder.

Youths with bipolar disorder are more severely ill and have higher genetic loading than adult-onset cases (3–5). Furthermore, children's brains are typically free from confounding factors known to affect brain structure and function (e.g., extensive treatment, substance use, history of electroconvulsive therapy). Finally, studying children and adolescents facilitates observation during a time in development when there are hormonal shifts known to have neuromodulatory effects on brain regions such as the temporal lobe (6, 7). By virtue of all of these factors, there is an increased likelihood of uncovering significant brain anatomic abnormalities in this early-onset group.

Although no discrete brain area has been consistently reported abnormal in the adult bipolar disorder MRI (structural) literature, some data lend support to several proposed neuroanatomic models of emotion regulation (8–10). One of these proposed models includes the following brain regions: prefrontal cortex, amygdala-hippocampus complex, hypothalamus, thalamus, insular cortex, ventral striatum, and interconnected structures (8). The amygdala, hippocampus, and thalamus are of particular interest in the study of bipolar disorder because of their functional roles in the brain. For example, the amygdala is integral in emotion-related aspects of behavior, memory, and learning; the thalamus processes sensory information and integrates activity among forebrain regions; and the hippocampus plays a role in learning and memory in providing contextual information (11). Several prior structural MRI studies of adults with bipolar disorder have reported abnormalities in the limbic structures and thalamus (12–18). Abnormalities in these structures might confer a propensity toward dysregulated mood states and vulnerability toward developing a mood disorder.

Prior structural MRI studies in pediatric bipolar disorder have indicated that there are anatomic abnormalities

in a number of the structures implicated in the neural systems governing affective and cognitive processes. Botteron and colleagues demonstrated a loss of the normal asymmetry in the frontal lobe (19). A study conducted by Friedman and colleagues (20), which included adolescents with schizophrenia and bipolar disorder in a combined patient group, found that this patient group had reduced intracranial volumes and increased frontal and temporal sulcal sizes relative to healthy subjects. A third report that used the same subjects as in the Friedman et al. study found the patient group had reduced thalamic area relative to healthy subjects (21). A recent study consisting predominantly of adults with bipolar disorder included a subgroup of 14 adolescents (age range=10–22 years). These youths had smaller hippocampal and amygdalar volumes than did 23 healthy adolescents (18). Finally, DelBello and colleagues (22) recently reported that adolescents with bipolar disorder (N=23) had smaller amygdala and enlarged putamen volumes compared with healthy subjects (N=20). In summary, prior MRI studies have suggested that youths with bipolar disorder have abnormalities in a number of the brain areas discussed by Soares and Mann (8) in their neuroanatomic model of emotion regulation: total cerebral volume, frontal lobe, hippocampus, amygdala, putamen, and thalamus. In order to assess anatomic findings in bipolar disorder further, we conducted a structural MRI study to evaluate brain volumes in early-onset bipolar disorder cases. We hypothesized that youths with bipolar disorder would have abnormalities in structures involved in the model of affect regulation discussed by Soares and Mann (8). In particular, the amygdala, hippocampus, and thalamus were chosen *a priori* on the basis of previous imaging studies of youths and adults with bipolar disorder.

Method

Subjects

The study was approved by institutional review boards at the Massachusetts General Hospital and McLean Hospital. Subjects were recruited through the McLean Hospital outpatient program and professional-patient advocacy groups. Inclusion criteria were DSM-IV diagnosis of bipolar disorder, age 6–16 years, and right-handedness. Male and female subjects of all ethnicities were recruited. Healthy subjects, all right-handed, were recruited through community newspaper advertisements and had no DSM-IV axis I diagnosis according to structured and clinical interviews and no family history of affective disorders or psychotic disorders in first-degree relatives. Exclusion criteria were major sensorimotor handicaps; full-scale IQ <70 or learning disabilities; history of claustrophobia, head trauma, loss of consciousness, autism, schizophrenia, anorexia or bulimia nervosa, electroconvulsive therapy, or alcohol or drug dependence/abuse (in the 2 months preceding the scan or a total history of 12 or more months); active medical or neurologic disease; metal fragments or implants; or current pregnancy or lactation.

Procedure

Seventy subjects (all outpatients) and their parents (or guardians) signed assent and informed consent forms. Three subjects

were determined ineligible during interview, and one stopped the study because of lack of interest. Sixty-six scans were obtained; three scans were unreadable due to motion artifact. Therefore, data from 63 subjects scanned as part of an ongoing neuroimaging study are included in this report: 43 youths with DSM-IV bipolar disorder and 20 healthy subjects.

All of the youths underwent a diagnostic semistructured interview (Schedule for Affective Disorders and Schizophrenia for School-Age Children—Epidemiologic Version [K-SADS-E] [23]) and a clinical interview by board-certified child psychiatrists (J.A.E., S.C.). In addition, parents were administered an indirect K-SADS-E regarding their children by trained raters. These B.A.-level raters received 4 months of training on the administration of the K-SADS-E under the supervision of senior raters and the senior investigator (J.B.). All raters had established a high degree of interrater reliability: from 175 interviews, the mean kappa was 0.90, and all disorders achieved kappa coefficients >0.82. Final DSM-IV diagnoses were established by the consensus diagnosis of clinical and structured interviews.

Each youth received a physical and neurological examination that included Tanner staging (a I–V scale of pubertal development) (24) and cognitive testing. The age at onset of each illness was determined by parental report of symptoms on the structured interview. Age at illness onset was defined as the time when the youth met full diagnostic criteria (e.g., age at onset of bipolar illness was the age at which the youth first met full diagnostic criteria for mania). Children and adolescents were given several subtests of the Wechsler Intelligence Scale for Children, 3rd ed. (WISC-III) (25) which permitted the estimation of verbal IQ. Handedness was assessed using the Edinburgh Handedness Questionnaire (26).

Measures of current psychopathology were obtained using the Young Mania Rating Scale (27) and Global Assessment of Functioning Scale (GAF) (DSM-IV, p. 32).

Antipsychotic doses (converted to chlorpromazine equivalents) (28, 29), as well as number and type (antipsychotic, antidepressant, stimulant, anticonvulsant, lithium) of psychoactive medications at the time of scan were used as clinical variables.

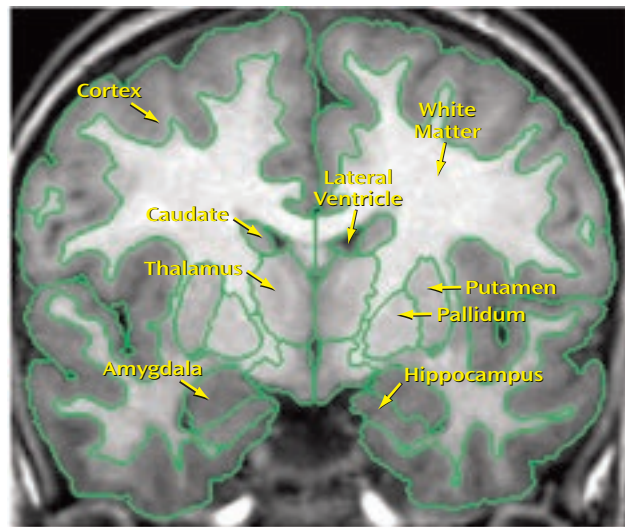
MRI Protocol

Structural imaging was performed at the McLean Hospital Brain Imaging Center on a 1.5-T Signa scanner (GE Medical Systems, Milwaukee). Acquisitions included a conventional T₁-weighted sagittal scout series (20 slices), a proton density/T₂-weighted interleaved double-echo axial series (120 slices, slice thickness=3 mm, field of view=24 cm², TR=3 seconds, TE=30/80 msec, acquisition matrix=256×192, number of excitations=0.5), and a three-dimensional inversion recovery-prepped spoiled gradient recalled echo coronal series, which was used for structural analysis (124 slices, prep=300 msec, TE=1 minute, flip angle=25°, field of view=24 cm², slice thickness=1.5 mm, acquisition matrix=256×192, number of excitations=2). All scans were reviewed by a clinical neuroradiologist to rule out gross pathology.

Image Analysis

Structural scans were transferred to the NMR Center for Morphometric Analysis-Charlestown Massachusetts General Hospital and coded and catalogued for blind analysis. Imaging analysis was done on Sun Microsystems, Inc. (Mountainview, Calif.) workstations using Cardviews software (30). The datasets were positionally normalized to overcome variations in head position by imposing a standard orientation on each scan using the midpoints of the decussations of the anterior and posterior commissure lines and the midsagittal plane at the level of the posterior commissure as points of reference for rotation and translation. The images were not rescaled to Talairach spatial dimensions in order

FIGURE 1. T₁-MRI Coronal Section at the Level of the Mamillary Bodies^a



^a Segmentation outlines shown in green.

to preserve individual and interhemispheric differences in the morphometry of structures (31).

The entire image sets were then segmented into gray, white, and CSF tissue classes by three image analysts, under the supervision of one of the authors (N.M.), all of whom had strong backgrounds in neuroanatomy and extensive training in morphometric analysis and who were blind to subject-identifying information. The segmentation method uses a semiautomated intensity contour algorithm for external border definition and signal intensity histogram distributions for delineation of gray-white borders (Figure 1). This technique allows for border definition as the midpoint between the peaks of the bimodal distribution for any given structure and its surrounding tissue (32).

Total cerebral volume. Segmentation of the regions of interest was performed following the anatomic definitions of Filipek and colleagues (33) for the total cerebrum. Total cerebral volume was defined as all gray and white matter in the cerebrum and did not include CSF, cerebellum, or brain stem. The cerebrum was measured across all 124 coronal slices in which it appeared.

Thalamus. Segmentation of the thalamus was performed following the anatomic definitions of Seidman and colleagues (34, 35) by tracing the trajectory of the hypothalamic fissure in the sagittal plane to separate the thalamus proper from the ventral diencephalon. The medial boundary of the structure was the third ventricle, and the lateral boundary was the internal capsule. The superior border was the body of the lateral ventricle, and the inferior border was the hypothalamic fissure.

Hippocampus and amygdala. The method of Filipek et al. (33) defines the amygdala and hippocampus as a continuous gray matter structure in the primary segmentation. These two structures are then separated from each other according to the procedure described by Seidman and colleagues (36) in which the hippocampus is separated from the amygdala at the rostral-coronal plane, where the hippocampus first appears. The segmentation of the amygdala was performed manually in its entirety, comprising approximately 11 subsequent coronal sections.

The coexistence of the amygdala and hippocampus in several coronal sections can make the precise identification of the ventral amygdalar border difficult (37). Therefore, we used the cross-referencing capability of the program Cardviews (38) to draw outlines delimiting the amygdala in axial and sagittal views (Figure 2

B, C); this preliminary procedural step allows a reliable separation of the amygdala from surrounding gray structures, such as the ventral part of the lentiform nucleus, the medial temporal cortex, and the hippocampus, thus eliminating the need to apply conventions to define the anterior amygdalar boundary (12, 22) or the amygdala-hippocampal junction (12). The anterior portion of the amygdala was segmented as it appears beneath the medial temporal cortex (slice K in Figure 2). At this region, the medial temporal cortex and the amygdala can give the impression of being only a thickening of the medial temporal cortex with no amygdala present, as has been reported previously (12, 22). Therefore, the definition of these borders was particularly aided by the tracing of cross-referenced outlines in the axial and sagittal planes (Figure 2 B, C). The choroidal fissure was used as the superior border of the amygdala along with the gray-white matter contrast between the amygdala and surrounding white matter. The gray-white matter contrast between the amygdala and its surrounding temporal white matter (consisting of the centrally located temporal white matter stem), as well as the gray-CSF contrast between the amygdala and the temporal horn of the lateral ventricle, was considered the lateral border of the amygdala. The parahippocampal cortex anteriorly, the brain exterior at the inferior lip of the choroidal fissure, and partially the hippocampus posteriorly were assigned as the medial borders of the amygdala. Finally, the inferior border consisted of the gray-white matter contrast between the amygdala and its surrounding temporal white matter anteriorly and by the alveus (of the hippocampus) and the temporal horn of the lateral ventricle posteriorly.

The volumes for each structure were derived by multiplying the number of voxels assigned to each structure on each slice by the voxel volume (the product of slice thickness and the square of in-plane resolution), followed by summing across all slices in which the structure appeared; the volumes are reported in cm³ (31).

For the reliability study, 10 scans from our data set were selected at random and blindly segmented by two raters. Five of the scans were also remeasured in a random order by one of the raters to estimate the intraclass correlation coefficient.

The standard interrater intraclass correlation coefficient for the total cerebrum was 0.93, and the intra- and interrater correlation coefficients, respectively, for the regions of interest were 0.88 and 0.84 for the amygdala, 0.95 and 0.96 for the thalamus, and 0.93 and 0.94 for the hippocampus.

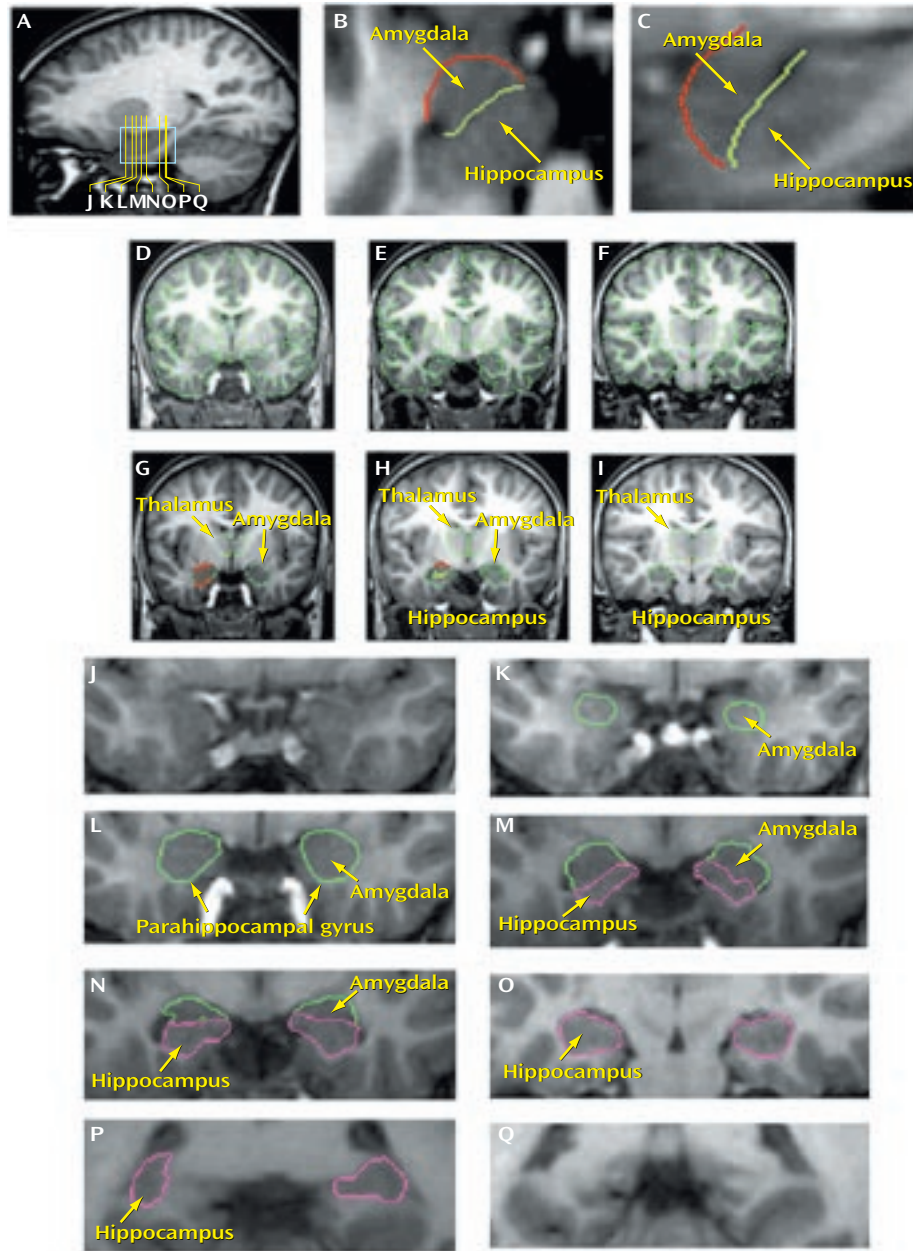
Data Analyses

S-Plus 6.0 (Insightful Corp., Seattle) was used for statistical analysis. All statistical tests were two-sided with alpha set at 0.05.

Differences in demographic and clinical variables were measured using *t* tests for continuous variables and chi-square tests for categorical variables. In addition, Pearson's correlations were calculated for clinical variables of the bipolar disorder group and those structures that differed significantly between bipolar disorder youths and healthy subjects. The clinical variables included Young Mania Rating Scale and GAF scores, number of psychoactive medications, chlorpromazine equivalents, and verbal IQ.

Noting that brain structure sizes possess variances that increase with their means, we analyzed our volumetric data on the natural logarithmic scale as a step toward uncoupling this non-Gaussian relationship. We conducted an exploratory multivariate analysis of variance (MANOVA) on the three-dimensional ensemble of log total hippocampal, amygdalar, and thalamic volumes with the effects of log total cerebral volume, age, sex, diagnosis, and the sex-by-diagnosis interaction controlled. In addition, an exploratory MANOVA analysis was performed to assess the effects of age group, mood state, medication type, and presence of ADHD or psychosis on the log hippocampal and log total cerebral volumes.

We then proceeded to analyze the effects of these covariates on log brain structure sizes by three separate univariate linear regres-

FIGURE 2. Segmentation of the Amygdala, Hippocampus, and Thalamus^a

^a The segmentation method of the amygdala, hippocampus, and thalamus is shown in T₁-weighted MR images. In particular, the segmentation method used for the amygdala (developed by N.M.) is shown in detail in images J–P. Image A shows a sagittal slice passing through the hippocampus and amygdala. Yellow vertical lines show the locations of representative coronal sections J–Q. The blue box in A indicates the region containing amygdala (K–N), amygdala-hippocampal transition, anterior hippocampus (L), and the temporal-polar region rostral to the amygdala (J). B shows an axial section (the position of which is indicated by arrow in A) used for the tracing of an outline to separate the most anterior portion of the amygdala from the surrounding medial temporal cortex (red line). C shows an enlarged version of the blue box in A to emphasize the amygdala-hippocampal transition. In both B and C, the light green line represents the border between amygdala and hippocampus. In C, the red line shows the superior, anterior, and inferior limits of the amygdala. These outlines are used as guidelines to complete segmentation on the coronal plane (D–I). Similarly, the thalamus is segmented based on the intensity contrast between this structure and its surrounding white matter; a border delimits its inferior border at the hypothalamic fissure. Structural details from coronal slices J–O are enlarged to show the method of amygdala segmentation. J shows a rostral slice in the temporal lobe where the amygdala is not present. More caudally, the anterior part of the amygdala appears within the rostromedial temporal area beneath the cortex as shown in K. Further posteriorly, the middle portion of the amygdala occupies a position superior and lateral to the anterior parahippocampal gyrus as shown in L. Progressing in the rostrocaudal dimension, the hippocampus appears as shown in M and N, in which the amygdala is also present. In a more posterior location (O), there is only hippocampus as amygdala is no longer present. In P, the posterior most segment of the hippocampus is shown flanked laterally by the atrium of the lateral ventricle. Finally, Q is the coronal posterior to the hippocampus. To emphasize the amygdala and hippocampal outlines, the segmentation outlines of the other brain structures were omitted from images J–Q.

TABLE 1. Demographic and Clinical Characteristics of Pediatric Patients With Bipolar Disorder and Healthy Comparison Subjects

Characteristic	Bipolar Patients (N=43)		Healthy Subjects (N=20)	
	N	%	N	%
Sex				
Male	23	53.5	12	60.0
Female	20	46.5	8	40.0
Caucasian	42	97.7	18	90.0
	Mean	SD	Mean	SD
Age (years)	11.3	2.7	11.0	2.6
Handedness score (right minus left)	15.7	9.3	16.9	5.1
Height (cm)	147.1	14.2	142.2	15.2
Weight (kg) ^a	49.6	14.2	41.1	15.5
Head circumference (cm)	54.3	1.4	54.8	1.8
Tanner stage	2.5	1.7	2.0	1.6
Hollingshead socioeconomic status rating	48.9	13.0	54.4	17.2

^a Significant between-group difference ($t=-2.0$, $df=61$, $p=0.05$).

sion models. Employing our preliminary data summaries and linear regressions, we found no significant laterality effects on any of the volumetric outcomes; hence, we did not include brain hemisphere effects in our model selection procedure. We fit a linear regression model for log total hippocampal volume that contained the same effects as in the preliminary MANOVA. We also analyzed the data using the absolute volumes of the structures and the inferential findings were identical to the log scale analyses.

Results

Data from 63 subjects are included: 43 youths with DSM-IV bipolar disorder (mean age=11.3 years, $SD=2.7$; current episode: mixed=52.3%, manic=15.9%, depressed=11.4%, euthymic=20.5%) and 20 healthy comparison subjects (mean age=11.0 years, $SD=2.6$). Characteristics of the groups are summarized in Table 1. There were no significant height, head circumference, or Tanner stage differences between groups; there was a significant difference in weight (Table 1).

Although the bipolar disorder youths had verbal IQ scores in the normal range, they were significantly lower than those of comparison subjects (mean=100.7 [$SD=14.3$] versus 116.9 [$SD=12.7$]; $t=4.3$, $df=61$, $p<0.001$). The bipolar disorder group also scored lower on the GAF (mean=49.5 [$SD=6.0$] versus 68.8 [$SD=1.9$]; $t=19.0$, $df=61$, $p<0.001$). The bipolar disorder subjects had a number of comorbid conditions (mean=7.2, $SD=3.2$). The most common comorbid conditions were oppositional defiant disorder (67% [$N=29$]) and ADHD (51% [$N=22$]). Most bipolar disorder youths (86% [$N=37$]) had experienced at least one episode of major depression (mean age at onset=6.8 years, $SD=3.6$), and 40% ($N=17$) had a history of psychosis. One youth had a history of alcohol abuse, which occurred more than 3 months before enrollment. Medications used at the time of MRI included lithium (26% [$N=11$]), anticonvulsants (42% [$N=18$]), antidepressants (30% [$N=13$]), stimulants (21% [$N=9$]), atypical antipsychotics (76% [$N=33$]), and other

(19% [$N=8$]), which included anticholinergics and beta-adrenergics. Table 2 presents details of the clinical and treatment characteristics of the bipolar disorder patients.

Clinical neuroradiological interpretations of the scans showed normal variants in three healthy subjects (slightly prominent lateral ventricles [$N=1$], large cisterna magna [$N=1$], and pineal cyst [$N=1$]) and two bipolar disorder subjects (mildly prominent lateral ventricles [$N=1$] and large cisterna magna [$N=1$]). One healthy subject had findings that were atypical but of unclear clinical significance (a tiny focus of hypointensity in the subcortical white matter of the superior left frontal lobe). Nine bipolar disorder subjects had findings that were atypical and again of unclear clinical significance (prominent ventricles [$N=3$], right-greater-than-left asymmetry of the temporal horn [$N=2$], multiple white matter hyperintensities in the bilateral parietal area [$N=1$] and in the left hemisphere [$N=1$], nonspecific punctate T_2 foci in the left parietal region [$N=1$], and bilateral widened perivascular spaces noted in the inferolateral portion of the basal ganglia [$N=1$]).

Volumetric Measurements

Table 3 contains the limbic and thalamic volume data. The amygdala volumes were measured across a mean of 12.1 slices ($SD=1.6$, range=9–15) on the right side, and 11.5 slices ($SD=1.4$, range=8–15) on the left. The hippocampal volumes were measured across a mean of 24.4 slices ($SD=1.7$, range=21–28) on the right side, and 24.7 slices ($SD=2.0$, range=20–29) on the left. The thalamic volumes were measured across a mean of 22.7 slices ($SD=1.3$, range=18–25) on the right side, and 22.9 slices ($SD=1.1$, range=21–25) on the left.

No significant correlations were seen between any clinical variables and the hippocampus or total cerebral volume for the bipolar disorder group.

When the bipolar disorder group was assessed using the exploratory MANOVA analysis, we found no significant effects of age group, mood state, medication type, or presence of ADHD or psychosis on volume in the hippocampus or cerebrum.

The initial exploratory MANOVA of the three-dimensional brain structure ensemble yielded potentially significant effects for sex ($F=2.76$, $df=3$, 55, $p=0.05$) and the sex-by-diagnosis interaction ($F=2.17$, $df=3$, 55, $p=0.10$).

Hippocampus. The linear regression model for log total hippocampal volume contained the same effects as in the preliminary MANOVA. We found a possible sex-by-diagnosis interaction in raw hippocampal volumes by sex and diagnosis (Table 3). Although not significant, we retained age in the model, since this covariate was fixed by our study design; main effects and the interaction of sex and diagnosis remained significant when age was dropped. We tried controlling for height and weight but found that neither of their effects was significant; the sex-by-diagnosis interaction remained significant. We found significant effects of log cerebral volume, sex, diagnosis, and the sex-

TABLE 2. Clinical and Treatment Characteristics of Pediatric Patients With Bipolar Disorder

Characteristic	Pediatric Bipolar Patient Group					
	Total (N=43)		Boys (N=23)		Girls (N=20)	
	Mean	SD	Mean	SD	Mean	SD
Age at onset of bipolar disorder (years)	7.0	3.8	5.8	3.6	8.8	3.8
Age at onset of ADHD ^a	4.8	2.4	4.3	2.3	6.0	2.4
Duration of bipolar disorder (years)	2.8	3.1	2.8	2.7	2.9	4.2
Young Mania Rating Scale Score ^b	24.8	8.0	22.6	8.2	28.0	6.9
Age at first hospitalization ^c	6.4	5.0	4.5	4.0	8.6	5.6
Chlorpromazine equivalents at time of study ^d	117.1	98.0	121.6	100.5	111.5	97.6
Number of psychoactive medications at time of study ^e	2.1	1.1	2.3	1.1	2.1	1.0
	N	%	N	%	N	%
History of hospitalization	14	32.6	7	30.4	7	35.0

^a Total N=22.^b Significant gender difference ($t=2.1$, $df=41$, $p=0.04$).^c Total N=14.^d Total N=33 (77%).^e Includes atypical antipsychotics, antidepressants, sedatives, mood stabilizers, and stimulants.

TABLE 3. Limbic and Thalamic Volumes of Pediatric Patients With Bipolar Disorder and Healthy Comparison Subjects

Brain Area and Patient Group	Bipolar Disorder				Healthy Subjects			
	N	Mean (cm ³)	SD	Coefficient of Variation (%)	N	Mean (cm ³)	SD	Coefficient of Variation (%)
Amygdala								
Boys	23	3.48	0.52	14.9	12	3.69	0.68	18.4
Girls	20	3.16	0.36	11.4	8	3.46	0.43	12.4
Total	43	3.33	0.47	14.2	20	3.60	0.59	16.5
Hippocampus								
Boys	23	7.61	0.70	9.3	12	7.87	0.87	11.0
Girls	20	6.83	0.75	11.1	8	8.16	0.62	7.7
Total	43	7.24	0.82	11.3	20	7.98	0.77	9.7
Thalamus								
Boys	23	16.36	0.88	5.4	12	16.87	1.18	7.0
Girls	20	15.02	0.99	6.6	8	16.15	1.05	6.5
Total	43	15.74	1.14	7.3	20	16.58	1.16	7.0

by-diagnosis interaction (Table 4). We pursued the source of interaction by fitting separate regression models of the same form to the boys and the girls. This step confirmed our pooled variance assumption when fitting the single model with a sex effect (39). We conclude that the sex-by-diagnosis interaction is being driven by the smaller hippocampus of the girls after log total cerebral volume and age are controlled.

Amygdala and thalamus. The same logarithmic transformations and model selection procedure was applied to the thalamic and amygdalar volumes separately. No significant effects of sex, diagnosis, or their interaction were found.

Discussion

The youths in our study with bipolar disorder had significantly smaller total hippocampal and total cerebral volumes. These findings contribute to the existing literature on bipolar disorder youths, which has shown loss of normal frontal lobe asymmetry (19), reduced intracranial volume (20), increased frontal and temporal sulcal size (20), reduced thalamic area (21), larger putamen volume

(12, 22), and smaller amygdala (18, 22) and hippocampal (18) volumes relative to healthy subjects.

These findings in children and adolescents differ somewhat from those in adults, but comparison of MRI results across studies is difficult because of variations in methodology. For example, until recently, it was difficult to even reliably measure structures such as the hippocampus—and particularly the amygdala—on MRI scans (12, 22). It is of note that the quality of the T₁-weighted images used in the present study, as well as the outlines traced on the cross-referenced axial and sagittal slices, allow reliable visualization and segmentation of the amygdala, eliminating the need to apply conventions for defining either the anterior amygdalar boundary (12, 22) or the amygdala-hippocampal junction (12). Despite the differences in the methodologies used across studies, it is worth highlighting the anatomic variations that have been reported in adult structural MRI studies relative to the findings reported here and in other pediatric studies of bipolar disorder patients.

Of the structures included in our *a priori* hypothesis, the thalamus and amygdala have varied findings in adult and child and adolescent studies. Several adult bipolar disorder studies have reported that both structures are in-

TABLE 4. Total Cerebral Volume, Age, Sex, Diagnosis, and Sex-by-Diagnosis Interaction Effects on Limbic and Thalamic Volumes in Pediatric Patients With Bipolar Disorder and Healthy Comparison Subjects

Brain Area and Covariate	Estimated Effect	SE	t (df=61)	p
Amygdala				
Log total cerebral volume	0.872	0.272	3.209	0.002
Age	0.002	0.007	0.229	0.82
Sex	0.019	0.064	0.299	0.77
Diagnosis	-0.013	0.062	-0.206	0.84
Sex-by-diagnosis	-0.003	0.076	-0.040	0.97
Hippocampus				
Log total cerebral volume	1.068	0.150	7.134	<0.001
Age	-0.001	0.004	-0.212	0.84
Sex	-0.090	0.035	-2.558	0.02
Diagnosis	-0.086	0.034	-2.529	0.02
Sex-by-diagnosis	0.100	0.042	2.385	0.02
Thalamus				
Log total cerebral volume	0.593	0.097	6.143	<0.001
Age	0.005	0.003	1.832	0.08
Sex	0.022	0.023	0.957	0.35
Diagnosis	-0.024	0.022	-1.098	0.28
Sex-by-diagnosis	0.022	0.027	0.807	0.43

creased (12, 13, 15), whereas two others reported smaller volumes: one reported smaller left amygdala (14) and the other smaller bilateral amygdalar volumes (18). In adolescents, one study found reduced thalamic area (although the patient group also included youths with schizophrenia) (21). Blumberg and colleagues (18) and DelBello and colleagues (22) have recently reported decreased amygdalar volumes in adolescents with bipolar disorder. In our study, youths with bipolar disorder had a nonsignificant but possibly meaningful decrement in thalamic volumes compared with healthy subjects; our current study size may be underpowered to detect this effect, and we continue to study this structure as more scans are acquired. However, we found that there were no differences in amygdalar volume between groups.

Interpreting the differences between adult and pediatric findings depends on our understanding of the phenomenology of mood disorders across the lifespan: if early- and adult-onset bipolar disorder are the same illness, then age at onset may differentially affect brain structure. Alternatively, childhood- or adolescent-onset bipolar disorder may be a distinct disorder from adult-onset illness with a different set of neuroanatomic correlates. Cross-sectional measures of anatomic volume also may not adequately reflect the progressive and regressive patterns of growth that affect different brain structures at various stages of development (e.g., the subcortical gray matter structural changes that occur in the temporal lobe during the pre-teen years [38, 40]).

Our finding of decreased hippocampal volumes is the first report of this finding in children and adolescents with bipolar disorder and further extends the findings of Blumberg and colleagues in which bilateral hippocampal volume reductions were seen in adolescents with bipolar disorder but not in adults with the illness (18). Adult bipolar disorder studies of hippocampal volumes have found ei-

ther decreased or normal volumes (12, 16, 18, 41). It is possible that the adult studies in which reduced hippocampal volumes were reported may have included at least some adults who had childhood-onset illness. However, this information was not included in those studies. If age at onset, particularly childhood-onset, is related to reduced hippocampal volumes, this could partially explain the mixed results seen in adult studies. Our finding of reduced hippocampal volume, as well as another group's similar finding among adolescents with the illness (18), may represent a finding unique to early-onset bipolar disorder. This finding in our youths is of interest in light of neuropathologic studies of the hippocampus in bipolar disorder, which have suggested there may be abnormal neurodevelopment and remodeling of synapses in that structure (42–48).

A study of adults that compared monozygotic twins discordant for bipolar disorder found that the right hippocampus was smaller in the sick twin compared with the well twin (17), suggesting that this finding might be a structural correlate for the presence of disease. Given that reduced hippocampal volume was the only significant limbic finding in our group of bipolar disorder youths, and that at least two studies in adults (16, 17) had a similar finding in the right hippocampus, this may also be a finding reflective of disease.

Female bipolar disorder subjects showed a more pronounced decrease in hippocampal volume than did male bipolar youths relative to their comparison subjects. This sex-by-diagnosis interaction could reflect a variety of factors. For example, girls may need to have more significant structural abnormalities in order to reach threshold for disease expression. Alternatively, since the girls with bipolar disorder in our study had significantly higher Young Mania Rating Scale scores than the boys, the smaller hippocampal volumes in girls might be reflective of a greater degree of psychopathology. However, both male and female bipolar disorder youths had reduced hippocampal volumes, and it may be that there is some abnormality in the hippocampus, reflective of disease, that expresses itself more robustly in girls than in boys (49).

In our study we found that total cerebral volume was smaller in bipolar disorder youths (mean=5.4%, SD=6.4%); smaller total cerebral volume has also been reported in four prior studies of bipolar disorder youths (19–21, 50). Most adult bipolar disorder studies, including the recent study published by Blumberg and colleagues (18), have not found differences in total cerebral volume. Therefore, the difference seen in total cerebral volume between early- and adult-onset bipolar disorder cases relative to healthy groups suggests that affected youths may have distinct neurodevelopmental trajectories, possibly from having brains that have developed differently or from early apoptotic pruning of neuronal circuits. A smaller total cerebral volume in early-onset bipolar disorder illness may be reflective of a neurodevelopmental phenomenon. It should be noted that there is evidence of nonuniform scaling

across the brain regions studied herein relative to global changes in total cerebral volume (51).

Although abnormal anatomy does not necessarily confer abnormal function, some structural differences may increase the risk of developing dysfunction. Such differences might also be markers of preexisting susceptibility or vulnerability and could aid in identifying different patient phenotypes (52). Bipolar disorder youths often have behavioral and developmental difficulties early in life (2, 52–54), and their symptoms of bipolar disorder typically begin between the ages of 5–11 years (1, 3, 55); in our study the mean age at onset was 7.0 years (SD=3.8). Many of the progressive and regressive events in the brain, particularly in the temporal lobe, occur during this age range, and alterations in normal brain development during this time may result in the symptoms of bipolar disorder.

Adult genetic and neuroimaging studies in both schizophrenia and bipolar disorder lend support for a multifactorial etiology; one possibility is of a “two-hit” hypothesis, a genetic predisposition in combination with environmental influences, resulting in the disorder (35, 36, 56, 57). For example, our finding of smaller hippocampal volumes may reflect developmental or genetic influences or environmental insults (e.g., hypoxia) that have led to reductions in what were previously normally developing volumes. This hypothesis suggests that two hits (genetic and environmental), occurring either independently or in interaction, may result in reduction of hippocampal volumes as well as in the expression of bipolar disorder.

This report describes to our knowledge the largest MRI study of pediatric bipolar disorder to date. However, the number of subjects was still relatively small and represents only a cross-sectional look at youths with bipolar disorder and comparison subjects. Our findings should be considered in light of other limitations, such as the smaller number of comparison subjects relative to the number of youths with bipolar disorder, the lack of rating scales for depressive symptoms, and the difficulty in reliably determining the age at onset of a child or adolescent's mood instability based on parental recall. Although we did examine the effects of several clinical variables, including age and type of medication as well as chlorpromazine equivalents, and found no significant effects, the power in our study may have been insufficient to fully assess the possible effects of these parameters.

Our findings support the hypothesis that the hippocampus may be involved in the underlying pathophysiology of pediatric bipolar disorder and may represent a unique characteristic of early-onset presentation of the disorder due to a genetic diathesis or derangements in growth processes around the time of illness onset. However, in order to obtain a better sense of the trajectory of abnormalities in this structure in bipolar disorder populations, longitudinal studies are needed. Future studies need large enough sample sizes of both sexes and a sex-matched healthy

comparison group to sort out diagnostic, age at onset, developmental, and sexual influences on these structures over time (6, 38, 58). A larger group of subjects is currently being accrued in order to further assess these findings with greater power and to assess the sexual dimorphism of other brain structures.

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