Reduced Amygdalar Gray Matter Volume in Familial Pediatric Bipolar Disorder

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ABSTRACT

Objective: Subcortical limbic structures have been proposed to be involved in the pathophysiology of adult and pediatric bipolar disorder (BD). We sought to study morphometric characteristics of these structures in pediatric subjects with familial BD compared with healthy controls. **Method:** Twenty children and adolescents with BD I (mean age = 14.6 years, four females) and 20 healthy age, gender, and IQ-matched controls underwent high-resolution magnetic resonance imaging at 3 T. Patients were mostly euthymic and most were taking medications. Amygdala, hippocampus, thalamus, and caudate volumes were determined by manual tracings from researchers blinded to diagnosis. Analyses of covariance were performed, with total brain volume, age, and gender as covariates. **Results:** No differences were found in the volumes of hippocampus, caudate, and thalamus between subjects with BD and controls. Subjects with BD had smaller volumes in the left and right amygdala, driven by reductions in gray matter volume. Exploratory analyses revealed that subjects with BD with past lithium or valproate exposure tended to have greater amygdalar gray matter volume than subjects with BD without such exposure. **Conclusions:** Children and adolescents with early-onset BD may have reduced amygdalar volumes, consistent with other studies in this population. Prolonged medication exposure to lithium or valproate may account for findings in adults with BD of increased amygdalar volume relative to controls. *J. Am. Acad. Child Adolesc. Psychiatry,* 2005;44(6):565–573. **Key Words:** magnetic resonance imaging, bipolar disorder, amygdala.

Neuroimaging studies of adults with bipolar disorder (BD) have indicated that subcortical structures may be involved in the pathophysiology of this condition (Strakowski et al., 2000). Primarily, candidate regions have included the hippocampus, caudate, putamen, thalamus, and amygdala. These limbic and paralimbic structures have all been implicated in circuits of mood regulation (Blumberg et al., 2002; Mayberg, 1997), making them logical candidates for further study in BD.

Examining subcortical morphology in children with BD is important for interpreting findings from adult studies in a developmental context. Pediatric, or early-onset BD, may represent a distinct subtype of BD because it has a phenomenology that is usually distinct from adult-onset BD. For example, children with BD have much higher rates of rapid cycling and irritability than adults (Findling et al., 2001; Geller et al., 2000). High comorbidity with attention-deficit/hyperactivity disorder (ADHD), conduct disorder, and oppositional defiant disorder also is unique to early-onset BD (Wilens et al., 2003). Because few studies have examined the longitudinal course of these children into adulthood, it is not clear whether pediatric-onset BD is in all cases continuous with adult mania or represents a unique disorder. Therefore, children with BD may or may not share neurobiological characteristics with adults who have BD. If BD is indeed continuous across the life

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cycle, then studying the neurobiological characteristics of pediatric BD and comparing findings with those of adults with BD could shed light on early trait characteristics as well as developmental changes in BD that occur with time.

Morphometric analyses of subcortical structures in patients with BD have yielded varying results. Studies have reported increased thalamic volume in adults (Dewan et al., 1988; Dupont et al., 1995) and youths (Frazier et al., 2002) with BD compared with controls. However, decreased thalamic volumes in adolescents with BD have also been reported (Dasari et al., 1999), whereas other studies have found no neuroanatomical differences in the thalami of adults with BD (Caetano et al., 2001; Dolan et al., 1990; Strakowski et al., 1993).

Most studies have found no differences in striatal volumes in BD (Aylward et al., 1994; Brambilla et al., 2002; Swayze et al., 1992). However, one study reported increased caudate volumes in men with BD (Aylward et al., 1994). Another study found increased caudate nuclei volumes in both BD and discordant (healthy) monozygotic twins compared with control monozygotic twin pairs (Noga et al., 2001). Age and illness duration were related to left putamen volume loss in subjects with BD, particularly in those with BD I (Brambilla et al., 2001). The only study to date examining striatal volumes in pediatric BD reported increased putamen volume and no differences from controls in caudate volume (DelBello et al., 2004).

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. Right hippocampal volume appeared decreased in monozygotic twins with BD compared with their discordant (healthy) twins (Noga et al., 2001). In a cohort of adolescents and adults with BD, hippocampal volume tended to be decreased compared with controls (Blumberg et al., 2003).

Finally, the amygdala may be a particularly relevant structure to study in BD. In addition to having a prominent role in emotion processing and response, the amygdala has been reported by some investigators to demonstrate activation abnormalities during functional imaging studies of adults with BD (Drevets et al., 2002; Yurgelun-Todd et al., 2000). Volumetric findings in adults with BD have been equivocal, with reports of similar (Swayze et al., 1992), decreased (Blumberg et al., 2003; Pearlson et al., 1997), or increased (Altshuler et al., 2000; Strakowski et al., 1999) amygdalar volumes. Amygdalar findings in pediatric BD are more consistent, as two studies of adolescents with BD found decreased amygdalar volumes in patients compared with healthy controls (Blumberg et al., 2003; DelBello et al., 2004). A third study involving adolescents and young adults with BD reported a trend to 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).

We wished to investigate the morphometry of relevant subcortical structures in children and adolescents with BD. Because of an ongoing high-risk study, we studied the offspring of parents with BD, thus enriching our cohort for familial early-onset BD. We hypothesized that we would find reduced amygdalar and hippocampal volumes and increased caudate and thalamic volumes in these children. We also investigated the additional potential role of mood stabilizer treatment in affecting subcortical brain morphology.

METHOD

This protocol was approved by the Stanford University Panel of Medical Research in Human Subjects. Twenty patients and 20 healthy volunteers were recruited from an ongoing study of offspring with BD and from the community. After obtaining oral and written informed consent from parents and oral and written assent from their offspring, semistructured interviews were conducted. Inclusion criteria for subjects with BD were age 9–18 years, biological parent with BD I or II, and diagnosis of BD I. 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.

Patients had at least one parent with BD I or II as diagnosed by the Structured Clinical Interview for *DSM-IV* Axis I disorders (SCID) (First et al., 1995), administered by a trained master's degree–level clinician (D.I.) and/or board-certified child and adolescent psychiatrist (K.C.). All subjects, patients and healthy volunteers, were evaluated by the affective disorders module of the Washington University in St. Louis Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS) (Geller et al., 1996, 2001) and the K-SADS-Present and Lifetime (K-SADS-PL) (Kaufman et al., 1997). Subjects were evaluated either by a board-certified child and adolescent psychiatrist (K.C.) or a trained master's degree–level research assistant (D.I.). Diagnostic decisions were ultimately made by a child and adolescent psychiatrist (K.C.) based on personal interview, discussion with the research assistant, and written notes of parental and subject responses to individual K-SADS questions. Current and lifetime diagnoses were established according to *DSM-IV* criteria. Age at onset of BD was determined retrospectively as the earliest period to the closest month that patients met criteria for a manic episode, as defined by the *DSM-IV*.

Healthy volunteers did not have a *DSM-IV* psychiatric diagnosis, had both parents without any diagnosis by SCID, and did not have

a first- or second-degree relative with BD as determined by the Family History Research Diagnostic Criteria (Andreasen et al., 1977).

Subjects were all outpatients at the time of magnetic resonance imaging (MRI). Patients with BD were administered the clinician-rated Young Mania Rating Scale (Fristad et al., 1995; Young, 1978), and they completed the Children's Depression Inventory (Kovacs, 1985), with help of a parent if they were younger than 12 years old. Patients with BD had psychostimulants discontinued for 24 hours before the scan, primarily due to a concurrent, separate functional MRI study of attention. They were allowed to continue any other current medications such as mood stabilizers or antidepressants due to the risk of mood destabilization. Medication history was obtained from direct interview with subjects and parent and review of medical records when available. Positive past exposure to lithium (Li) or valproate (VPA) was recorded if the subject had at least 6 months of treatment with either agent at standard doses or serum levels.

Magnetic resonance images of each subject's brain were acquired with a Signa 3-T 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 msec, TE = 6 msec, flip angle = 45 degrees, 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 × 0.9 × 1.2 mm^3. The images were reconstructed as a 124 × 256 × 256 matrix with a 1.5 × 0.9 × 0.9 mm^3 spatial resolution.

Imaging Processing and Measurement

Image data were imported into the program BrainImage 5.29 (Stanford Psychiatry Neuroimaging Laboratory; http://spnl.stanford.edu) for semiautomated image processing and quantification. After image importation, MRI data were corrected for bias field artifact, nonbrain tissue was removed using a semiautomated process, and the brains were positionally normalized to a stereotactic space (Talairach and Tournoux, 1988). To specify regional differences, each brain was divided into lobes with a semiautomated stereotactic-based parcellation method (Kates et al., 1999). The brains were divided into cerebral lobes, cerebellum, and lateral ventricles based on the rater' identification of three anchor points: the anterior commissure, the posterior commissure, and a midsagittal point above the axis created by the first two points. Raters who conducted morphometric analyses were blind to the diagnosis of each subject. Voxels comprising brain tissue were then segmented into gray matter, white matter, and CSF using a semiautomated fuzzy tissue segmentation algorithm (Reiss et al., 1998).

The total brain volume (TBV) was calculated by adding the cerebrum total tissue, cerebellum tissue, brainstem tissue, cerebrum total CSF, cerebellum CSF, ventricle box CSF, and brainstem CSF. Total cerebral volume was calculated by adding cerebrum total tissue, cerebrum total CSF, and ventricle box CSF. Total brain tissue was calculated by adding cerebrum total tissue, cerebellum tissue, and brainstem tissue.

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

Thalamus. Thalami were traced starting on the slice where the structure was first visible and followed until thalamic gray matter disappeared; the border between the gray matter of the thalamus and the surrounding white matter was used to outline the thalamus.

Caudate. Caudate nuclei were outlined on a specifically created subcortical gray matter stack with an automatically set pixel intensity threshold to make outlining consistent and reliable. Caudate was traced starting on the slice where the structure first appeared and outlined until it was no longer visible.

Hippocampus. 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 an inferior border of the hippocampus, medial border was defined by CSF and by the pons, where present, and the lateral border was marked by CSF or white matter tracts on the lateral edge of the hippocampus.

Amygdala. 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.

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

Statistical Analysis

Independent *t* tests were used in comparisons of subjects with BD and healthy controls for demographic variables and TBV. Brain structure volume data were first examined for normality to conform to the assumptions of the parametric statistics employed. One-way analyses of covariance were used for comparisons of brain structure volumes, with age and TBV as covariates. A p value of .05 (two tailed) was chosen as the significance threshold.

RESULTS

The cohort has been described elsewhere (Chang et al., in press) and is summarized in Table 1.

There were no significant differences between subjects with BD and controls in TBV (1484.34 ± 128.60 cm^3 (SD) versus 1522.48 ± 110.12 cm^3 (SD); $df = 37$, $F = 0.88$, $p = .35$). However, we covaried for TBV when analyzing subcortical volumes due to high correlation between TBV and total cerebral gray and white matter volumes. Subjects with BD had similar thalamic total (left plus right) volume (16.14 ± 1.14 cm^3 (SD) versus 16.43 ± 1.50 cm^3 (SD); $df = 36$, $F = 0.00003$, p = .99) and similar caudate total volume (9.51 ± .81 cm^3 (SD) versus 9.38 ± 1.33 cm^3 (SD); $df = 36$, $F = 0.971$, $p = .33$) compared to those of the control group.

No between-group difference was present for the hippocampus (3.62 ± 0.55 cm^3 (SD) versus 3.65 ± .50 cm^3 (SD); $df = 36$, $F = 0.001$, $p = .98$ for the left hippocampus and 3.74 ± .42 cm^3 (SD) versus 3.56 ± 0.45 cm^3 (SD); $df = 36$, $F = 1.90$, $p = .18$ for the right hippocampus).

TABLE 1
Demographics of Subjects

	Bipolar	Controls	*p*
No.	20	20	
Mean age, yr (SD)	14.6 (2.8)	14.1 (2.8)	0.59
Gender, % male	80	80	
Socioeconomic status (SD)	4.2 (0.8)	4.5 (0.7)	
Race (%)			
African-American	1 (5)	0 (0)	
Hispanic	1 (5)	2 (10)	
Asian	0 (0)	3 (15)	
White	18 (90)	15 (75)	
I/Q (SD)	109.5 (11.4)	116.0 (9.5)	0.06
Handedness (% right)	95	95	
Comorbid diagnoses (%)			
Attention-deficit/ hyperactivity disorder	16 (80)	0 (0)	
Anxiety disorder	7 (35)	0 (0)	
Oppositional defiant disorder	11 (55)	0 (0)	
Young Mania Rating Scale	15.4 (8.7)	—	
Children's Depression Inventory	15.3 (8.7)	—	
Children's Global Assessment Scale	54.3 (8.0)	—	
Past Psychotropic Medication Exposure (%)			
Stimulants	60	0	
Tricychantidepressants	15	0	
Selective serotonin reuptake inhibitors	65	0	
Atypical antidepressants	50	0	
Lithium	35	0	
Valproate	50	0	
Antipsychotics	35	0	
Any mood stabilizer	70	0	

Total amygdalar volume was decreased in subjects with BD compared with controls (10.4% decrease in volume overall, 3.93 ± .40 cm^3 (SD) versus 4.39 ± 0.45 cm^3 (SD); $df = 36$, $F = 10.02$, $p = .003$). Both left (2.00 ± 0.31 cm^3 (SD) versus 2.24 ± 0.35 cm^3 (SD); $df = 36$, $F = 4.26$, $p = .046$) and right amygdala (1.94 ± 0.20 cm^3 (SD) versus 2.15 ± 0.25 cm^3 (SD); $df = 36$, $F = 7.58$, $p = .009$) were significantly smaller in subjects with BD. Because of this difference, we sought post hoc to determine whether gray or white matter reduction in the amygdala was contributing to this finding. This decrease was driven by gray matter reduction in the right amygdala (9.1% decrease, 1.78 ± 0.19 cm^3 (SD) versus 1.96 ± 0.23 cm^3 (SD); $df = 36$, $F = 5.91$, $p = .02$; Fig. 1); amygdalar white matter was not significantly different between the two groups (Table 2).

Subjects with BD were divided into those who had and had not been treated with Li and/or VPA (n = 11 versus 9). Four of the subjects had been treated with both Li and VPA, four only with VPA, and three only with Li. A multivariate analysis of covariance covarying for age and TBV revealed significant differences between groups (subjects with BD treated with Li/VPA, subjects with BD not treated with Li/VPA, controls) in total amygdalar gray volume ($df = 2$, $F = 4.71$, $p = .016$). Follow-up one-way analyses of covariance revealed significant differences between the subjects with BD without Li/VPA exposure and controls ($df = 1$, $F = 10.1$, $p = .0039$) and a trend toward differences between the subjects with BD with and without Li/VPA exposure ($df = 1$, $F = 4.04$, $p = .06$) (Fig. 2). As an exploratory analysis, no effect of Li/VPA exposure was observed in the gray matter of other subcortical structures (Tables 3 and 4).

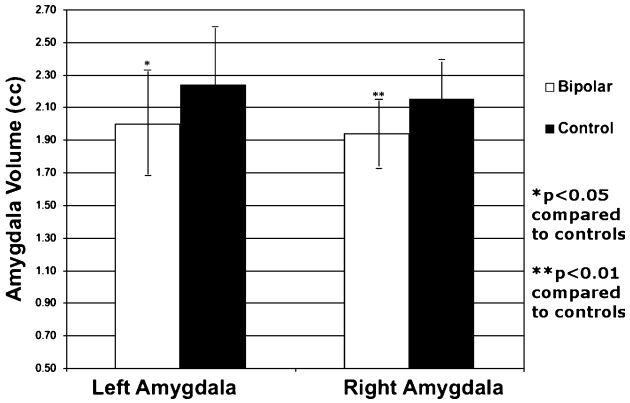

Fig. 1 Amygdala volumes in total bipolar subjects (n = 20) and healthy controls (n = 20).

DISCUSSION

We found children and adolescents with familial BD to have significantly smaller amgydalae than healthy controls, predominantly on the right and driven by gray matter reduction. No other subcortical structures examined (hippocampus, thalamus, and caudate) were different in volume between subjects with BD and controls. Furthermore, exposure to Li or VPA treatment appeared to confer protection against volume decreases in the left amygdala only.

Both human and animal studies strongly suggest that the amygdala contributes to emotion-related processes by attaching affective significance to perceived stimuli (Amaral et al., 1992). Monkeys with bilateral lesions of the amygdala show decreased emotional reactivity when presented with previously feared objects such as

TABLE 2
Brain Regional Volumes in Subjects With Biopolar Disorder and Controls

Brain Region	Bipolar cm^3 (SD)	Control cm^3 (SD)	p^a
Total brain volume (covaried by age)	1484.3 (128.6)	1522.475 (110.1)	.35
Total cerebral volume (covaried by age)	1291.4 (118.0)	1327.5 (100.0)	.34
Total cerebral gray matter	676.62 (62.26)	707.17 (52.26)	.09
Total cerebral white	458.10 (45.40)	471.15 (53.73)	.91
Total cerebral tissue	1134.73 (99.75)	1178.32 (92.14)	.18
Left amygdalar gray	1.85 (0.30)	2.03 (0.36)	.10
Left amygdalar white	0.15 (0.07)	0.20 (0.10)	.09
Right amygdalar gray	1.78 (0.19)	1.95 (0.23)	.02
Right amygdalar white	0.16 (0.07)	0.20 (0.07)	.19
Left amygdalar tissue	2.00 (0.31)	2.24 (0.35)	.046
Right amygdalar tissue	1.94 (0.20)	2.15 (0.25)	.009
Total amygdalar tissue	3.93 (0.40)	4.39 (0.45)	.003
Left thalamus	8.07 (0.59)	8.18 (0.76)	.73
Right thalamus	8.07 (0.59)	8.26 (0.77)	.77
Total thalamus	16.14 (1.14)	16.43 (1.50)	.99
Left hippocampus	3.62 (0.55)	3.65 (0.50)	.98
Right hippocampus	3.74 (0.42)	3.56 (0.45)	.18
Total hippocampus	7.35 (0.90)	7.22 (0.85)	.49
Left caudate	4.67 (0.46)	4.56 (0.72)	.26
Right caudate	4.83 (0.45)	4.82 (0.65)	.51
Total caudate	9.51 (0.81)	9.38 (1.33)	.33

[a] One-way analyses of covariance, with age and total brain volume as covariates.

snakes (Amaral, 2002), and human patients with amygdalar damage show deficits recognizing emotional facial expressions (Adolphs et al., 1999). The amygdala may be involved in positive and negative emotions. For example, it is sensitive to novelty (Schwartz et al., 2003) and may be involved in perceiving the reward value of stimuli (Baxter and Murray, 2002). Finally, the animal model of amygdala-kindled seizures bears resemblance to the development and progression of BD in humans, and both conditions may have similar neurobiological underpinnings (Post, 2004).

Neuroimaging studies in children and adolescents with BD have so far supported the amgydala's role in this disorder. In pediatric BD, this is the third report of decreased amygdalar volume from studies using high-resolution MRI data. Blumberg et al. (2003) reported a decrease in amygdalar volume of 15.6% compared with controls in a combined group of adolescents and adults with BD. DelBello et al. (2004) reported a

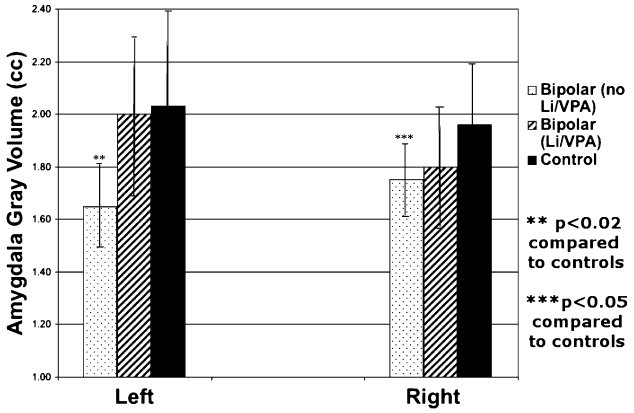

Fig. 2 Amygdalar gray volumes in bipolar subjects not exposed to Li/VPA, bipolar subjects exposed to Li/VPA, and healthy controls.

10.9% decrease in overall amygdalar volume in children and adolescents with BD, similar to our finding of a 10.4% decrease. Furthermore, another study found a trend for decreased left amygdalar volume (mean decrease, 13.6%) in adolescents and young adults with BD (Chen et al., 2004).

There are multiple possible etiologies for decreased amygdalar volumes in patients with BD. First, decreased volume may indicate an inborn trait, representing a genetically determined core neurobiological feature of BD. Second, decreased amygdalar volume may be the result of neurotoxic processes. Siegle et al. (2003) showed that decreased amygdalar volume in adults with unipolar depression was associated with increased amygdalar activity, suggesting that chronic amygdalar hyperreactivity could decrease amygdalar volume through toxic levels of glutamate neurotransmission.

Decreased glial density could also account for loss of amygdalar volume. In a postmortem study, adults with major depressive disorder, but not subjects with BD, were found to have reduced amygdalar glial density compared with control samples (Bowley et al., 2002). However, a subgroup of subjects with BD who did not have a history of Li or VPA exposure did have significantly decreased amygdalar glial density. Other reasons for decreased amygdalar size might include abnormal pruning during development. It is less likely that vascular or white matter changes would account for our findings, as volume differences were driven by gray matter decreases.

Past studies of adults with BD had reported decreased or unchanged amygdalar volume in adults with BD, but in these studies either thick-slice MRI (Pearlson et al.,

TABLE 3
Brain Region Volumes in Subjects with Bipolar Disorder Exposed to Li/VPA and Subjects With Bipolar Disorder Without Exposure

Brain Region	Bipolar With Li/VPA Exposure cm^3 (SD) (n = 11)	Bipolar Without Li/VPA Exposure cm^3 (SD) (n = 9)	p
Total cerebral gray matter	671.67 (55.99)	682.66 (72.20)	.079
Right amygdalar gray matter	1.80 (0.23)	1.75 (0.14)	.29
Left amygdalar gray matter	2.00 (0.30)	1.65 (0.16)	.057
Right hippocampal gray matter	3.39 (0.45)	3.20 (0.30)	.44
Left hippocampal gray matter	3.28 (0.56)	3.27 (0.25)	.76
Right thalamus gray matter	4.14 (0.43)	4.26 (0.57)	.44
Left thalamus gray matter	5.12 (0.49)	4.83 (0.74)	.82
Right caudate gray matter	3.66 (0.27)	3.79 (0.44)	.15
Left caudate gray matter	3.48 (0.39)	3.40 (0.41)	.93

Note: Li/VPA = Lithium/Valproate.

TABLE 4
Brain Regional Volumes in Subjects with Bipolar Disorder Without Li/VPA Exposure and Controls[a]

Brain Region	Bipolar cm^3 (SD) (n = 9)	Control cm^3 (SD) (n = 20)	p
Total cerebral gray matter	682.66 (72.20)	707.17 (52.26)	.56
Right amygdalar gray matter	1.75 (0.14)	1.96 (0.23)	.02
Left amygdalar gray matter	1.65 (0.16)	2.03 (0.36)	.04
Right hippocampal gray matter	3.20 (0.30)	3.15 (0.42)	.75
Left hippocampal gray matter	3.27 (0.25)	3.32 (0.44)	.84
Right thalamus gray matter	4.26 (0.57)	4.20 (0.57)	.60
Left thalamus gray matter	4.83 (0.74)	4.88 (0.63)	.59
Right caudate gray	3.79 (0.44)	3.64 (0.51)	.11
Left caudate gray	3.40 (0.41)	3.26 (0.60)	.27

[a] Eleven subjects with bipolar disorder were excluded: four with Li/VPA exposure, three with Li exposure, four with VPA exposure.

1997) or area of amygdala from one to two slices were used (Swayze et al., 1992). More recent studies using thin-slice volumetric measurements have indicated larger amygdalae in adults with BD compared with healthy controls (Altshuler et al., 2000; Strakowski et al., 1999), while another found decreased amygdalar volumes in adults with BD (Blumberg et al., 2003). Our findings agree with the latter study but at first glance appear contradictory to the findings of increased amygdalar volumes in adults. While the volume of the human amygdala normally grows with age as a normal part of brain development (Durston et al., 2001; Yurgelun-Todd et al., 2003), growth usually continues only through early adolescence. However, there are several possible reasons for this discrepancy. Although thought to be neurotoxic, chronic stress might also increase amygdalar volumes. Rats exposed to prenatal stress showed increased volume of the lateral amygdaloid nucleus compared with controls (Salm et al., 2004). There is also the possibility that increased activity may lead to increased blood flow and hypertrophy of amygdala in patients with BD. Thus, longer durations of illnesses in adults might lead to larger than normal amygdalae.

Psychotropic medications may also affect amygdalar volume. Chronic administration of Li or VPA in rats increases thyrotropin-releasing hormone binding in the amygdala, suggesting that the amygdala may be directly affected by mood-stabilizing medication (Pekary et al., 2004; Sattin et al., 2002) and possibly protected from any damaging effects of illness. This is consistent with our finding that subjects with previous Li and/or VPA treatment had larger amygdalae than those without. Li has been associated with gray matter increases in humans (Moore et al., 2000), prevention of stress-induced decrease in hippocampal dendrites in rats (Wood et al., 2004), and protection of neural progenitor cells from apoptosis (Shimomura et al., 2003). VPA has been reported to increase in vitro transcription of neurotrophic proteins (Bcl-2), inhibit neurotoxic proteins involved in neuronal death (GSK-3B), and activate protein kinases that stimulate neural dendritic growth (Manji and Lenox, 2000). Such medication effects may account for findings of larger amygdalae in previous studies in adults with BD that did not include assessment of past exposure to specifically Li or VPA. The positive correlation of age with amygdalar volume in adolescents and young adults with BD reported by Chen et al. (2004) also supports a developmental trajectory that

could explain the findings of increased amygdalar volumes in adults with BD. Li by itself was not found to affect amygdalar volume, but the effects of other mood stabilizers such as VPA were not examined in this study, and long-term administration of these agents might lead to increased amygdalar gray matter and volume.

It is also not known what effect chronic substance abuse, less common in pediatric subjects, has on brain structure size. Due to chronic substance use being less common in pediatric BD, and the fact that children usually have relatively shorter duration of medication exposure than adults, neuroimaging studies in pediatric BD may yield ''cleaner'' results. Finally, it is possible that pediatric-onset BD is a distinct and separate disorder from adult BD. Although retrospective studies suggest that pediatric and adult BD are continuous (Lish et al., 1994; Perlis et al., 2004), no definitive prospective data are available to support this suggestion. Thus, two such distinct disorders might have different neurobiological characteristics.

While the relationship between brain structure size and function has not been definitively established, decreased volume may indicate abnormal function of that structure (Siegle et al., 2003). The amygdala has high connectivity with orbitofrontal, ventrostriatal, and dorsal prefrontal regions, forming circuits that are thought to regulate mood and contribute to the pathophysiology of BD (Blumberg et al., 2002; Chang et al., 2004). Functional neuroimaging studies have reported abnormalities in amygdalar activation in adults with BD (Drevets et al., 2002; Yurgelun-Todd et al., 2000). Therefore, relatively smaller amygdalae in children and adolescents with BD might indicate abnormally functioning limbic circuits contributing to mood dysregulation.

Although subcortical structures such as the hippocampus, thalamus, and caudate are involved in limbic circuitry and may contribute to the pathophysiology of BD, we did not find differences in volumes of these structures in our subjects with BD compared with controls. Reports of volumetric findings in these structures in both adults and adolescents with BD have been highly inconsistent. Thus, we add to the literature failing to show morphometric abnormalities in the hippocampus, thalamus, and caudate. Given the equivocal findings in these other subcortical structures, it is all the more compelling that this is the third consecutive study to report decreased amygdalar volumes in pediatric BD.

Limitations

Our study is limited by several factors, foremost the presence of psychotropic medication exposure in most of our subjects. Although we attempted to control for Li or VPA exposure, many of our subjects had taken antidepressants, antipsychotics, and other anticonvulsants, of which the long-term effects on brain structure are unknown. Many of our subjects also had past stimulant exposure for which we could not control. Most of our cohort also had ADHD. No studies of patients with ADHD have specifically examined amygdalar volumes, so the contribution of this syndrome to our findings is unknown. Finally, our cohort is unique in that each subject with BD had at least one first-degree relative (parent) with BD, and each healthy control had no first-degree relative with a psychiatric disorder and no known family history of BD. This enrichment of our cohort with highly familial BD combined with our obtaining a highly ''clean'' control group may have enhanced our ability to detect neurobiological differences between groups but limited the ability to generalize our findings to all pediatric patients with BD.

Clinical Implications

Although neuroimaging is not yet a practical tool for diagnosing BD, there are several implications from this work that are of clinical relevance. First, identification of such a consistent brain abnormality, that of reduced amygdalar volume, in children with BD helps to validate BD as a distinct psychiatric diagnosis in children (Robins and Guze, 1970). This finding and other biological findings in this population may aid in decreasing the stigma for such externalizing disorders (such as blaming parents for their children's manic behaviors) and inform clinicians that structural abnormalities in limbic structures, and not simply willful behavior, may lead to severe mood dysregulation. Second, while preliminary, our findings of relatively increased amygdalar gray matter in patients with significant past exposure to mood stabilizers provide further understanding of how these medications may work to stabilize mood in BD. Furthermore, this finding supports other work suggesting that medications such as Li and VPA offer neuroprotection and that chronic administration of these medications might lead to biological benefits that could outweigh potential long-term adverse effects. Future neuroimaging studies of patients, followed prospectively

from childhood (ideally before the onset of fully developed BD) to adulthood would aid in discerning vulnerability traits from illness and medication effects and contribute further to the understanding of how morphometric brain abnormalities are associated with the development and course of BD.

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