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Original Article

Volumetric reductions in the subgenual anterior cingulate cortex in adolescents with bipolar I disorder

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Objectives: A range of prefrontal and subcortical volumetric abnormalities have been found in adults and adolescents with bipolar disorder. It is unclear, however, if these deficits are present early in the onset of mania or are a consequence of multiple mood episodes or prolonged exposure to medication. The goal of this study was to examine whether youth with bipolar I disorder who recently experienced their first episode of mania are characterized by brain volumetric abnormalities.

Methods: Anatomical images from magnetic resonance imaging of 26 13- to 18-year-old adolescents with bipolar I disorder and 24 age-comparable healthy controls with no personal or family history of psychopathology were analyzed using whole-brain voxel-based morphometry (VBM).

Results: Compared with healthy controls, adolescents with bipolar I disorder had significantly less gray matter volume in the left subgenual cingulate cortex [$p < 0.05$, family-wise error (FWE)-corrected].

Conclusions: Adolescents with a recent single episode of mania have smaller subgenual cingulate cortex volume than do their healthy counterparts, suggesting that this anomaly occurs early in the onset of, or may predate the disorder. Longitudinal studies are needed to examine the impact of this volumetric reduction on the course and outcome of this disorder.

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Bipolar disorder (BD) is characterized by dysfunction in emotion and cognition (1) that includes cycling mood states ranging from depression to mania and impulsivity. Affected individuals may show excessive goal-directed and pleasure-seeking behavior during manic episodes and reduced hedonic capacity during depressive episodes, all of which have a high potential for morbidities ranging from substance use to suicide (2). Over time, this pattern of polarized positive and negative shifts in emotion may increase the vulnerability to recurrence of symptoms between episodes or during periods of stress (3, 4). Deficits in cognitive and emotional processing may occur alongside a course of bipolar illness that may indicate specific neural abnormalities that may predict poor long-term outcome (5). Magnetic resonance imaging (MRI) has been used as a noninvasive tool to detect aberrant brain structure and function *in vivo*. The use of MRI has been critical in integrating clinical assessment of symptoms that may be associated with dysfunction in emotion and cognition and biologically mediated brain abnormalities in BD.

In this context, there is growing evidence of structural, functional, and neurophysiological abnormalities associated with BD in interconnected

brain regions, including portions of the prefrontal cortex and subcortical, medial temporal, and cerebellar structures (6). It is likely that some of these anomalies occur very early in the development of the syndrome (7–9). In fact, findings from recent studies suggest that clinical (10) and neurobiological (11, 12) characteristics of patients experiencing their first episode of mania are different from those of multi-episode patients or of patients with chronic mania. Specifically, as compared to those with multiple episode histories, first-episode BD patients appear to be younger, with a lower body mass index (BMI), a higher incidence of past or current cannabis abuse, and higher severity of illness (10). First-episode BD individuals appear to show reduced total intracranial and white matter volumes relative to controls (12), whereas multi-episode adults with BD appear to show larger lateral ventricles relative to first-episode adults with ventriculomegaly being associated with a higher number of prior manic episodes (11). Prior multi-episode studies usually performed in adults have reported conflicting findings and are limited by confounding factors such as mood state, comorbidities, and pharmacological treatments. Importantly, up to two-thirds of adults report that the onset of their symptoms occurred during adolescence (13). Even studies that have suggested early prefrontal abnormalities in children at familial risk for BD (14) have not been able to localize the differences to a specific region in the prefrontal cortex.

All of these factors have limited our ability to relate structural abnormalities to specific behavioral manifestations that characterize bipolar illness at its onset and over time. Besides symptoms of mania and depression, impulsivity has been shown to be a central feature early in and across the course of BD (15, 16). It may be measured as a dimension of illness in BD, but has rarely been correlated with a neurobiological measure. Understanding the relation between impulsivity and structural changes that might be occurring in adolescents with BD may increase our understanding of the pathophysiology of this condition.

The aim of the present study was to survey tissue volumes across large cortical regions using voxel-based morphometry (VBM) to evaluate volumetric differences between carefully characterized adolescents with bipolar I disorder who in their lifetime have only experienced a single episode of mania (BD-I) and a demographically comparable healthy control group (HC). Previous VBM studies in adults with multi-episode BD have found reductions in gray matter in the left ventromedial temporal cortex and bilateral cingulate cortex (17), in the anterior thalamus (18), or no significant volumetric differences between BD and healthy comparison groups (19). When multi-episode BD adults were parsed into medicated and unmedicated groups, the unmedicated group showed reductions in the posterior cingulate and superior temporal gyrus gray matter with corresponding white matter increases in these regions, and the medicated group showed gray matter reductions in the lateral orbital cortex (20). Children and adolescents with BD are presumed to have less illness burden by virtue of fewer lifetime manic and depressive episodes. One VBM study of children with bipolar I or II disorder found reductions in left dorsolateral prefrontal cortical gray matter (21). In many of these studies, the burden of illness on brain structure as indexed by illness severity and the number of manic episodes was not consistently or explicitly evaluated.

Based on these VBM studies as well as other structural MRI investigations in BD (6), we sought to determine whether, compared with HCs, adolescents with histories of a single episode of mania would show reduced gray matter volume in prefrontal and subcortical regions involved in the regulation of emotion, including the dorsolateral prefrontal and anterior cingulate cortices, striatum, and amygdala. In this single manic episode BD-I cohort, we predicted further that regional gray matter reductions would be correlated with indicators of illness burden including dimensional ratings of mania, depression, and impulsivity, and a prior history of depression. Based on results of the VBM whole-brain analysis, a subsequent assessment was conducted using manually traced morphometry to further define the location of volumetric abnormalities and to relate them to clinical characteristics.

Materials and methods

Participants

The university panel (Stanford University, Stanford, CA, USA) of medical research in human subjects approved this research protocol. After complete description of the study to the subjects and their parents, written informed consent was obtained from the parents, and written assent was obtained from youth under the age of 18. Twenty-six adolescents (ages 13 to 18 years) diagnosed with bipolar I disorder and as having their first manic episode within the previous 12 months were recruited by referral to a Pediatric Bipolar Disorders Clinic and from the surrounding community.

Participants in this group were assessed to ensure that in their lifetime they only had a single manic episode and were excluded if any subsequent manic episodes occurred prior to neuroimaging. Twenty-four healthy adolescents without any personal or family history of psychiatric diagnoses or exposure to psychotropic medications were recruited through advertisements in the local community. A telephone screening with a guardian established that participants were fluent in English, did not have any metal in their body, and had no history of head injury (with loss of consciousness over 5 min), seizures, or developmental or substance dependence disorders. Although a history of substance use was not an exclusion criterion for BD-I participants, they could not have had their manic episode in the context of substance use, and were required to be drug-free for at least one month prior to scanning. When indicated by clinical interview, a urine drug screen confirmed a participant's abstinence from recreational drugs prior to the scan. BD-I participants who were taking medications were washed out from stimulants 24 hours prior to neuroimaging. To avoid risk of mood destabilization, BD-I subjects were allowed to continue to take psychotropic medications including mood stabilizers, atypical antipsychotics, or antidepressants. Medication history was recorded and used in exploratory analyses.

Diagnostic and clinical assessments

After informed consent had been obtained, participants were evaluated for current and lifetime psychiatric disorders, using the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (22) for affective disorders and the Kiddie Schedule of Affective Disorders and Schizophrenia-Present and Lifetime Version (KSADS-PL) (23) for other psychiatric diagnoses, administered separately to parents and children by interviewers with established symptom and diagnostic inter-rater reliability (kappa > 0.9). A manic episode was defined by Diagnostic and Statistical Manual, 4th edition, Text Revision (DSM-IV-TR) criteria that lasted at least 1 week and could not have been precipitated by exposure to recreational drugs, antidepressants, psychostimulants, or other medication or medical conditions. Diagnoses were determined by a consensus conference attended by board-certified child and adolescent psychiatrists (MKS and KDC) and masters-level WASH-U-KSADS interviewers after both parent and child interviews had been completed. Symptom severity in BD-I subjects was assessed on the day of scan using the Young Mania Rating Scale (YMRS) (24), the Children's Depression Rating Scale-Revised Version (CDRS-R) (25), and the Childhood Global Assessment Scale (CGAS) (26) by raters with established symptom reliabilities [intra-class correlation (ICC) > 0.9]. Trait impulsivity was measured using the self-rated Barratt Impulsiveness Scale (BIS-11), which yielded subscale scores on the dimensions of attentional, motor, and nonplanning impulsivity (27). While age was noted on the day of scan, sex, socioeconomic status (Hollingshead Four Factor Index) (28), pubertal stage (Pubertal Development Scale) (29), IQ [Wechsler Abbreviated Scale of Intelligence (WASI)] (30), and handedness (Crovitz Handedness Questionnaire) (31) were assessed within 2 weeks prior to scan during a period of euthymia.

Imaging

All subjects were scanned on a 3T GE scanner (GE Healthcare Systems, Milwaukee, WI, USA). Anatomical images were obtained using a T1-weighted spoiled gradient-recalled acquisition in the steady state (SPGR) sequence with the following parameters: repetition time (TR) = 6.436 msec; echo time (TE) = 2.064 msec; flip angle = 15°, with an in-plane resolution of 0.859 mm × 0.859 mm in a 256 × 256 matrix and a slice thickness of 1.5 mm. Two subjects (one from each group) were excluded for motion artifacts causing severe distortion of the boundary between segmented gray and white tissue images.

Image analysis procedure

Data were analyzed using the default parameters of SPM8 (Wellcome Department of Cognitive Neurology, London, UK) with Matlab 7.8.0 (R2009a; MathWorks, Natick, MA, USA). A diffeomorphic image registration algorithm (DARTEL) (32) was used for image registration to a template generated from the total subject group. We followed the general image processing protocol outlined by Ashburner and Friston (33) that includes manually checking images for scanner artifacts and anatomical anomalies that would affect the image analyses and manually aligning images using the reorient tool in SPM8. Images were initially segmented using the segmentation algorithm in SPM8 (34). Using the DARTEL toolbox, we generated templates for image registration that were used to derive Jacobian scaled warped tissue class images for gray and white matter. These resulting modulated and warped images were then smoothed with an isotropic Gaussian kernel of 8 mm full-width at half-maximum and examined with an absolute

masking threshold of 0.05. The resulting images had a normalized voxel size of 1.5 mm^3.

Manual volumetric region-of-interest (ROI) measurements

Based on a previously published tracing protocol (35), anterior and posterior sections of the left and right subgenual anterior cingulate cortices (sACCs) were measured using manual tracings to confirm structural alterations identified in SPM8 by a rater who was blind to group membership. The rater (MM) achieved a high level of inter-rater reliability (ICC coefficients were ≥ 0.85 for all sACC subregions) with gold-standard drawings (35). BIJ software (http://spnl.stanford.edu/tools/brainimagej.htm) was used to trace, segment into gray, white, and cerebrospinal (CSF) partitions, and to measure total brain volumes (TBVs). sACC ROIs were drawn coronally with reference to a sagittal view using a spatially realigned gray-scale image, and gray matter volumes were measured on a segmented image with minimal CSF contributing to the gyrus. The sACC was drawn on two or three sagittal slices bound anteriorly and superiorly by the corpus callosum and inferiorly by the cingulate sulcus. The medial sACC boundary divided left and right subregions of this structure. The clear emergence of the putamen in its respective hemisphere marked the last slice of the anterior sACC. The posterior sACC began where the anterior sACC ended, and was posteriorly bound by the disappearance of the white matter tracts outlining the gyrus rectus. After tracing coronally, the ROIs were checked in the sagittal view for voxels that had unintentionally extended dorsally into the corpus callosum, or ventrally into the gyrus rectus. Gray matter volumes within each ROI were computed by BIJ software and exported into a spreadsheet for statistical analysis.

Data analysis

Statistical analyses were conducted on demographic and clinical data using Statistical Analysis System (SAS) software, version 9.1 (SAS Institute, Cary, NC, USA), and these data were first examined for normality using univariate analyses to conform to the assumptions of the parametric statistics employed (Shapiro-Wilks statistic, $W > 0.97$; $p > 0.21$). Nonparametric rank sum tests were used for any non-normal sampling distributions. Structural volumetric data constituted the dependent variable and group (BD-I versus HC) was the independent variable. Covariates in the statistical design for imaging data included participants' age, IQ, and TBV on segmented, unmodulated, and unsmoothed volumes to account for any undetected developmental effects on gray matter. Whole-brain *t*-tests were conducted on the smoothed, modulated, and segmented gray and white matter images with a voxel threshold of $p < 0.05$, family-wise error (FWE)-corrected, using additional nonstationary cluster extent correction (36) at that threshold. Contrasts were set for HC > BD-I and for BD-I > HC.

We conducted post-hoc exploratory analyses to examine the effects of a range of clinical variables on the VBM results. For all subjects, we extracted average voxel-wise gray matter values from ROI clusters that were significant in the primary whole-brain analysis using the MarsBar toolbox (http://marsbar.sourceforge.net/) and a threshold of $p = 0.001$, extent = 40 voxels uncorrected (37, 38). Extracted ROI values were correlated with clinical measures including CDRS, YMRS, and impulsivity. Using the smoothed, modulated, and segmented gray matter images, additional whole-brain *t*-tests were conducted within the BD-I group between the various mood states on the day of scan (determined by WASH-U-KSADS and YMRS and CDRS ratings, where manic state was confirmed by a YMRS score > 20, depressed state by a CDRS score > 40, mixed state by a YMRS score > 20 and CDRS score > 40, and euthymic state by a YMRS score < 10 and a CDRS score < 20), between BD-I participants with a prior history of major depressive disorder (MDD) and those with no history of MDD, between those with and without co-occurring conditions, between those with and without current or lifetime medication exposure, and between lithium-exposed and lithium-naïve BD-I participants. For these exploratory analyses, a signal threshold of $p < 0.001$ and extent threshold of 40 voxels were applied after adjusting for TBV, a threshold consistent with previous VBM analyses performed in BD samples (20). We corrected significance levels for multiple comparisons using a Bonferroni correction (0.05/3 comparisons = 0.017). Because the sample sizes for these subgroup analyses are smaller than the entire sample and exploratory, the results of these analyses should be interpreted with caution.

An analysis of covariance (ANCOVA) was used to analyze manually traced volumetric data in the sACC with group as the between-subject factor and age, intracranial volume, and IQ as statistical covariates, as was done for the VBM analysis. Associations between manual volumetric data and mania and depressive symptom ratings, along with

subject values of VBM generated extracted volumes (at a threshold of $p = 0.05$, FWE-corrected), were explored within the BD-I group using Spearman's correlations with a significance level of $p = 0.05$.

Results

Participant characteristics

Demographic and clinical characteristics of the BD-I and HC participants are presented in Table 1. There were no significant differences between the two groups in age [$t(48) = 1.72$], gender [$\chi^2(n = 50) = 0.34$], pubertal stage [$t(45) = 0.45$], socioeconomic status [$t(43) = 0.90$], race [$\chi^2(n = 50) = 7.8$], or handedness [$\chi^2(n = 50) = 1.92$] (all $p > 0.05$). HC adolescents had significantly higher WASI IQ scores than did the BD-I participants [$t(47) = 2.07$, $p = 0.04$]. On the day of their scan, 27% (n = 7) of the BD-I participants met criteria for mania, 31% (n = 8) were in a mixed state of mania and depression, 31% (n = 8) were depressed, and 12% (n = 3) were euthymic. As expected, BD-I adolescents reported significantly higher YMRS scores (mean = 19.9, SD = 7.4) than did the HC adolescents [mean = 0.17, SD = 0.48; $t(48) = 13.6$, $p < 0.01$]. Similarly, the BD-I participants had significantly higher scores on the CDRS [$t(48) = 9.0$, $p < 0.01$] and CGAS [$t(48) = 18.6$, $p < 0.01$] than did the HC participants. A total of 54% of adolescents with BD-I reported at least one prior depressive episode preceding the onset of their mania by an average of 2.8 years. Level of trait impulsivity was significantly higher in BD-I than in HC youth across all three subscales (all $p < 0.01$). Some BD-I teens also had other lifetime psychiatric diagnoses, including 38% with attention-deficit with hyperactivity (n = 10), 8% with generalized anxiety disorder (n = 2), 19% with oppositional defiant disorder (n = 5), 4% with conduct disorder (n = 1), and 19% with history of marijuana abuse (n = 5); 15% (n = 4) of the BD-I group had

Table 1 Demographic and clinical variables

Variable	Bipolar I disorder (n = 26)	Healthy controls (n = 24)
Age, years, mean (SD)	15.7 (1.6)	14.9 (1.4)
Gender, female, n (%)	13 (50)	14 (58)
Right handedness, n (%)	21 (81)	21 (88)
Socioeconomic status, mean (SD)	4.72 (0.54)	4.55 (0.69)
Self-assessed pubertal development, mean (SD)	3.25 (0.44)	3.18 (0.56)
Race, Caucasian, n (%)	18 (69)	14 (58)
Full-scale IQ, mean (SD)	106 (9.2)	112 (11.0)
YMRS score, mean (SD)[a]	19.9 (7.4)	0.2 (0.5)
CDRS score, mean (SD)[a]	43.2 (14.3)	17.8 (1.3)
CGAS score, mean (SD)[a]	54.5 (10.2)	93.6 (3.1)
BIS–attentional impulsivity, mean (SD)[a]	21.1 (5.3)	13.8 (3.3)
BIS–motor impulsivity, mean (SD)[a]	25.4 (4.5)	20.5 (3.4)
BIS–nonplanning impulsivity, mean (SD)[a]	29.0 (6.3)	24.2 (4.3)
Mood state (day of scan), n (%)[a]		
Manic	7 (27)	0 (0)
Mixed (manic + depressed)	8 (31)	0 (0)
Depressed	8 (31)	0 (0)
Euthymic	3 (12)	24 (100)
Co-occurring diagnoses, n (%)		
ADHD	10 (38)	0 (0)
Generalized anxiety disorder	2 (8)	0 (0)
Oppositional defiant disorder	5 (19)	0 (0)
Conduct disorder	1 (4)	0 (0)
Depressive episode before mania, n (%)	14 (54)	–
No. of months to scan after manic episode, mean (SD)	5.57 (3.4)	–
Lifetime medication exposure, n (%)[a]		
Lithium	8 (31)	0 (0)
Atypical antipsychotics	19 (73)	0 (0)
Antidepressants	9 (35)	0 (0)
Psychostimulants	7 (27)	0 (0)
Any medication	22 (85)	0 (0)
No. of weeks on meds on scan day[a]	16.8 (24.1)	0 (0)

ADHD = attention-deficit hyperactivity disorder; BIS = Barratt Impulsiveness Scale; CDRS = Childhood Depression Rating Scale; CGAS = Clinical Global Assessment Scale; SD = standard deviation; IQ = intellectual quotient; YMRS = Young Mania Rating Scale.
[a]$p < 0.05$.

mood-congruent psychotic symptoms during their first episode of mania. At the time of scan, about a third of BD-I participants (n = 8) had a lifetime exposure to lithium (mean exposure = 3.75 weeks), 73% (n = 19) were exposed to atypical antipsychotics (mean exposure = 4.1 weeks), 35% (n = 9) were exposed to antidepressants (mean = 3.6 weeks), and 27% (n = 7) were exposed to psychostimulants (mean exposure = 9.3 weeks); only 15% (n = 4) of the BD-I participants had never been exposed to any psychotropic medications.

Neuroimaging results

Images of brain structure acquired with MRI were first analyzed using VBM to give an unbiased and comprehensive assessment of anatomical differences throughout the brain (33). The HC and BD groups did not differ in total segmented gray matter volume [$t(48) = 0.11$], total segmented white matter volume [$t(48) = 1.62$], or TBV [$t(48) = 1.35$] (all $p > 0.05$) (Table 2). In whole-brain analyses, BD-I participants had significantly smaller gray matter volumes than did HC participants in the left sACC [Brodmann area (BA) 25sg, Talairach coordinates: $x = -8$, $y = 20$, $z = -14$] [$t(45) = 5.24$, $p = 0.019$ using a voxel significance threshold of $p < 0.05$, FWE-corrected with a nonstationary smoothness correction, cluster size $k = 13$] after covarying for age, IQ, and TBV (Fig. 1). Given evidence of sex-related differences in the anterior cingulate cortex in individuals with BD (39), group by sex analyses were performed and demonstrated no change in the whole-brain results. There were no significant group differences in white matter volume ($p > 0.05$, FWE-corrected), and no areas of gray matter volume in which the BD-I group had greater volume than the HC group ($p > 0.05$, FWE-corrected). Using a more liberal statistical threshold, whole-brain analyses revealed a larger contiguous region of volume reduction in the sACC (Talairach coordinates: $x = -8$, $y = 23$, $z = -15$) [$t(45) = 5.59$, $p = 0.001$ using a voxel significance threshold of $p < 0.001$, uncorrected with a nonstationary smoothness correction, cluster size $k = 899$]. No other volumetric reductions were identified at this uncorrected threshold.

Table 2. Volumetric assessment of adolescents with bipolar I disorder and healthy controls (without bipolar I disorder)

Volume (cm^3)	Bipolar I disorder (n = 26) Mean (SD)	Healthy controls (n = 24) Mean (SD)
White matter	524.24 (48.30)	514.40 (38.40)
Gray matter	761.57 (62.00)	759.50 (51.70)
Total brain (gray + white)	1285.82 (108.50)	1273.89 (87.00)

SD = standard deviation.

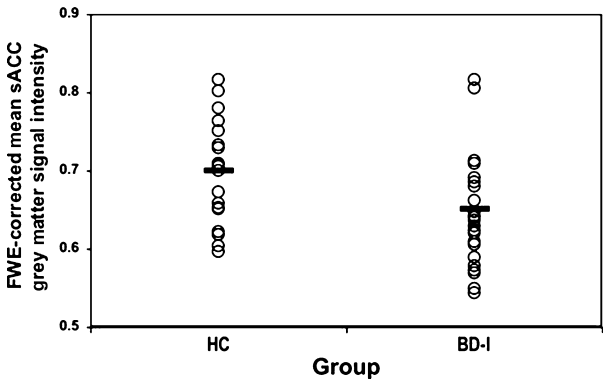

Fig. 1. Extracted mean subgenual anterior cingulate cortex (sACC) gray matter values in bipolar disorder (BD-I) and healthy comparison (HC) groups by voxel-based morphometry. FWE = family-wise error.

Within the BD-I group, YMRS, CDRS, and trait impulsivity subscales of motor, attention, and nonplanning scores were not significantly correlated with mean sACC extracted *t*-scores (lowest $p < 0.07$ for motor and attention impulsivity). Within the HC group, none of the correlations of trait impulsivity subscales, YMRS, and CDRS with sACC ROI average *t*-scores was significant, likely because of the restricted subclinical range of these scores within the HC group. Moreover, there were no significant differences within the BD-I sample involving mood state, history of prior depression, the presence of cooccurring conditions, or current or lifetime medication exposure (all whole brain *t*-tests $p > 0.05$). Using an uncorrected $p < 0.001$ and extent = 40 statistical threshold, lithium-exposed BD-I participants (n = 8) compared to a lithium-naïve subgroup (n = 18) showed increased gray matter volume in the left and right superior temporal gyrus [left BA 22: $t(23) = 4.29$, $p = 0.91$, cluster-wise FWE-corrected; right BA 22: $t(23) = 4.00$, $p = 0.91$, cluster-wise FWE-corrected] and left parietal lobule [BA 40: $t(23) = 4.84$, $p = 0.77$, cluster-wise FWE-corrected], and reduced gray matter volume in the right cerebellum [$t(23) = 3.93$, $p = 0.86$, cluster-wise FWE-corrected].

Total brain, age, and IQ adjusted means and standard deviations for the manually traced sACC measurements are presented in Table 3. ANCOVAs yielded no statistically significant group differences for the left anterior [$F(45) = 0.34$, $p = 0.56$], left posterior [$F(45) = 0.09$, $p = 0.77$], right anterior [$F(45) = 1.67$, $p = 0.20$], or right posterior

Table 3. Mean total brain volume and IQ- and age-adjusted subgenual anterior cingulate cortex (sACC) volumes of adolescents with bipolar I disorder and healthy control subjects with manual volumetry (cm^3)

	Bipolar I disorder (n = 26)	Healthy controls (n = 24)	Analysis	
Region	Mean (SD)	Mean (SD)	*F*	p-value
Left sACC	0.43 (0.08)	0.41 (0.05)	0.03	0.85
Left anterior sACC	0.27 (0.05)	0.25 (0.03)	0.34	0.56
Left posterior sACC	0.16 (0.03)	0.16 (0.02)	0.09	0.77
Right sACC	0.37 (0.04)	0.38 (0.02)	0.63	0.43
Right anterior sACC	0.21 (0.02)	0.24 (0.01)	1.67	0.20
Right posterior sACC	0.16 (0.02)	0.14 (0.01)	0.45	0.50
Overall sACC	0.81 (0.12)	0.79 (0.07)	0.26	0.61
Total brain volume (gray + white)	1357 (109)	1351 (83)	0.20[a]	0.84

SD = standard deviation.
[a]*t*-value.

[$F(45) = 0.45$, $p = 0.50$] subregions of the sACC. Adjusted mean left posterior sACC regions volumes were negatively correlated with YMRS ratings at a trend level within the BD group (Spearman's $r = 0.37$, $p = 0.06$). There were no statistically significant correlations between CDRS scores and any adjusted sACC volumes (highest $r = 0.29$, $p = 0.15$) within the BD-I group.

To further examine the relations between brain volumes measured using VBM and those computed using manual volumetry, average voxel-wise gray matter values were extracted using MarsBar at a threshold of $p = 0.05$, FWE-corrected, and correlated with sACC gray matter volumes traced manually. The FWE-corrected VBM-derived cluster correlated significantly with the manually traced left posterior sACC (Spearman's $r = 0.31$, $df = 50$, $p = 0.03$) across all subjects (Fig. 2). There were no other statistically significant correlations between VBM and manually derived sACC volumes (highest $r = 0.27$, $df = 50$, $p > 0.05$). Figure 3 illustrates the relation between the sACC volume difference derived from SPM8 and manually traced volumes.

Discussion

This study was designed to examine whole-brain volumetric differences between adolescents with a recent history of a single episode of mania and HCs. Our results indicate that, compared to demographically similar HCs, adolescents with BD-I are characterized by a significant reduction in left sACC volume. No other brain regions significantly differentiated the two groups of participants in gray or white matter volume, perhaps due to studying a relatively narrowly defined phenotypic sample of adolescents early in their course of bipolar illness, or due to the early influence of neurotrophic medication exposure which may have modified the neurodevelopmental trajectory of a severe illness onset.

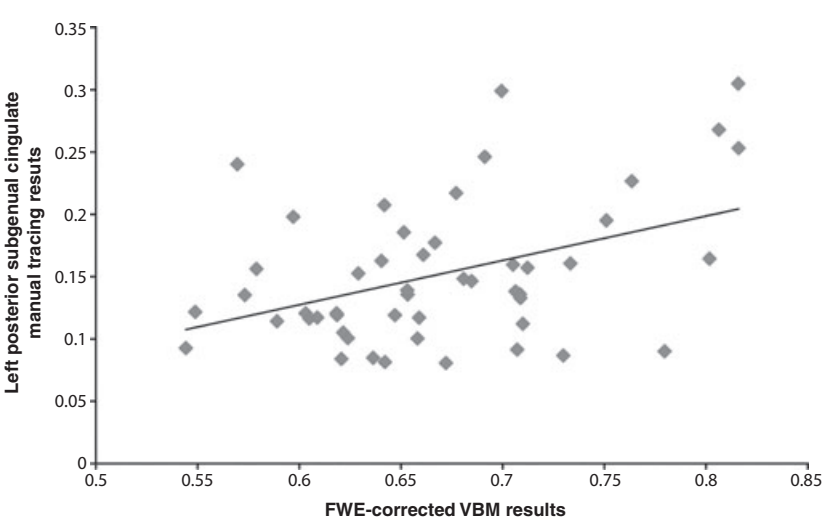

Fig. 2. Scatterplot of voxel-based morphometry (VBM) and manual tracing results across all subjects (Spearman's correlation, $R = 0.31$, $p = 0.03$). FWE = family-wise error.

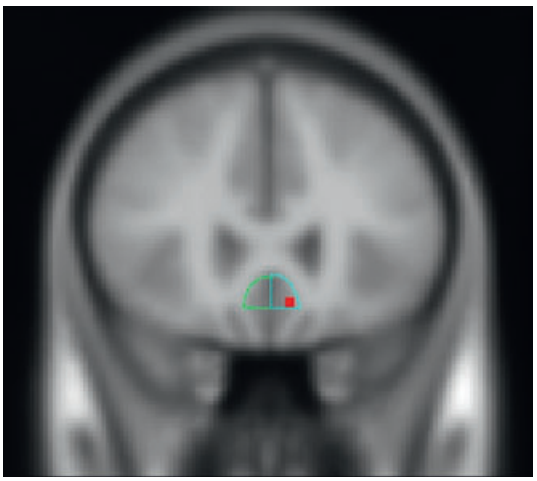

Fig. 3. Adolescents with bipolar I disorder have smaller subgenual cingulate gray matter volume than do healthy adolescents on whole-brain analysis [results shown in red at a statistical threshold of $p < 0.05$, family-wise error (FWE)-corrected and superimposed on manually traced left and right subgenual cingulate cortices].

The VBM finding of smaller sACC volume in adolescents with bipolar I disorder than in HCs extends the results of previous studies in pediatric BD that have also documented significant reductions in ACC volume. Studies using both manual tracing methods (40, 41) and VBM (42) have found reductions in anterior cingulate gyral volumes when comparing pediatric BD samples to typically developing children and to children with autism spectrum disorders (41). Given that these studies were conducted with pediatric samples, their results were unlikely to be confounded by many years of recurring mood episodes, medication exposure, or substance abuse. As such, this finding may represent a departure from typical gray matter pruning and maturation of the ventral and rostral regions of the ACC that occurs during adolescence (43, 44). Specifically, gray matter sACC volume loss in adolescents with mania relative to controls may suggest a developmentally anomalous level of pruning in a region functionally involved in the regulation of emotion. Our results therefore suggest a neural basis for symptoms associated with BD (45) that accounts for abnormal hedonic manifestations and impulsivity through disruption of healthy prefrontal cortical development.

Reductions in sACC gray matter have also been documented in a spectrum of mood disorders and demonstrate its functional role in emotional processing. For example, investigators have reported reduced sACC volume in children before and after the onset of pediatric bipolar illness (46), in individuals with first-episode affective psychosis with a positive mood family history (47), in psychotic major depression (35), in distinguishing first-episode BD from first-episode schizophrenia both cross-sectionally and longitudinally (48), and early in the course of mood disorders in adults (49). Psychosis was not a prominent feature of our sample, supporting a prior study that volumetric anomalies in sACC are less likely due to the presence of psychosis and more likely related to aberrant mood (48). The experience of negative mood states in healthy individuals has previously been reported to be associated with the sACC (50, 51). Moreover, the rich bidirectional connections between the sACC and key regions involved in emotional processing, including the amygdala, hippocampus, and parahippocampus (52, 53), implicate its functional role in processing emotion. Finally, neuropathophysiological changes such as glial cell reductions in the sACC have been found in individuals with anomalous emotional functioning, including persons diagnosed with BD (54).

Reversal of structural and functional impairments in the sACC by psychotropic medications has also provided some clues about the role of the sACC in the pathophysiology of BD. For example, lithium has shown neurotrophic effects by reversing sACC gray matter volume reductions in individuals with BD who respond to treatment (55), and youth with familial BD exposed to lithium and valproate had larger sACC volumes compared to those without such an exposure (56). Atypical antipsychotics have been found to be related to increases in sACC functional activation during working memory (57) and response inhibition (58) tasks. Our sample was relatively lithium-naïve, with an average lithium exposure of only 3.75 weeks (in eight participants), suggesting that there was limited neurotrophic benefit to our sample as a whole at the time of scan. Analyses comparing gray matter volumes in lithium-exposed and lithium-naïve BD participants did not yield statistically significant findings within the sACC (data not shown), but did indicate regional increases in the bilateral superior temporal gyrus, and left inferior parietal lobe. Longitudinal follow-up of our sample will aid in elucidating both the role of early intervention with lithium or other pharmacological interventions and the specificity of sACC gray matter restoration for a positive prognostic outcome.

Elevated impulsivity scores on the BIS-11 have been found to be associated with an early onset of illness, more frequent episodes, a history of suicide attempts, and a more severe course of illness in individuals with BD (59). Investigators using VBM have also shown that left rostral ACC gray matter volume is inversely correlated with both BIS total

scores and BIS motor impulsivity scores (60). In our study, BD-I adolescents were characteristically impulsive with higher BIS scores in attention, motor, and nonplanning impulsivity than the HC group, but these measures did not correlate significantly with sACC volumes. Importantly, sACC volumes were significantly smaller in the BD-I than in the HC group even after covarying for these dimensional scores, suggesting that this volumetric difference is impervious to functioning on these constructs. Although brain structure and function are not always directly correlated, increased activity in the sACC has recently been linked to harm avoidance in adolescents (61), suggesting that a disrupted sACC may play a role in risk-taking in individuals with BD. Further research is warranted to examine the functional significance of reduced sACC volumes in BD and whether sACC volumetric reductions will lead to increased severity, frequency, or duration of manic episodes or track with impulsivity over illness course.

Four prior studies found no evidence of significant reductions in sACC associated with BD. Sanches et al. (62) found no significant differences between 15 children and adolescents with BD (mean age = 15.5 years) and 21 healthy adolescents in subgenual prefrontal volumes using a semi-automated volumetric assessment. Hajek et al. (63) found no reduction in sACC in a combined sample of adolescents and young adults (ages 15–30), or in combined samples of bipolar I and bipolar II probands. Mitsunaga et al. (56) similarly did not find sACC differences in children and adolescents with familial BD compared to a healthy comparison group. Finally, Adler et al. (7) used VBM with a larger and slightly older sample (mean age 20) of 33 individuals per group and reported an increase in ACC gray matter volume in young adults with first-episode mania relative to HCs. There are several possible reasons for these inconsistent results. First, the wide range of developmental stages of the participants in prior studies may have contributed to their disparate findings. Small sample sizes, heterogeneous phenotypes (64), and variable levels of medication exposure (65) have limited previous studies in pediatric BD leading to potential type II error. In the present study, we attempted to reduce heterogeneity by sampling adolescents with a first episode of mania (bipolar I disorder only) who had few comorbidities and who were relatively medication naïve. Our results may thus represent a subtype of more classic adolescent onset mania that is characterized by early prefrontal structural anomalies.

We did not obtain consistent results in analyses using VBM versus manual tracing methods, likely due to the fact that VBM is a boundary-free method of analysis that provides information about areas within brain regions that maximally differ between groups, whereas manual volumetry relies on sulcal-gyral and other anatomical landmarks. Therefore, examining the entire sACC using manual volumetry may miss localized differences in the sACC observed by VBM. Correlations computed between manually traced volumes and extracted sACC volumes from VBM indicate that results of these two approaches are consistent in localized subregions of the sACC. In general, however, these methods appear to yield different findings, making it challenging to compare the results of studies that use different methods to measure brain volumes. Nevertheless, by utilizing both these methods in the present study, we generated important data that should be relevant for future group studies. Moreover, unlike previous VBM studies in pediatric BD which used ROI analyses (21, 42), our VBM whole-brain results indicate a group difference at a robust and corrected statistical threshold, suggesting additional studies exploring structural deficits in the sACC in bipolar disorder populations are warranted.

Significant differences in gray matter volume may be influenced by differences in the shape or location of a particular structure or area. In addition, our whole-brain analysis primarily revealed gray matter differences, but by using VBM we may have missed subtle differences in white matter densities, which have previously been identified in adolescents with BD by using diffusion tensor imaging (66), or in volume, which have previously been identified by morphometric studies in adults with BD (12, 20). However, by employing DARTEL, we were able to optimize our spatial normalization and registration accuracy compared to standard methods (67), increasing confidence that our results were driven by morphological differences rather than due to registration errors (68). Furthermore, although it is possible that the mood state of our participants on the day of scan affected our findings, we found no mood state-related morphometric associations in our sample, or indeed, in the literature. The effect of a previous depression may have also influenced our results, although our findings did not change after we controlled for history of depressive episodes. Finally, the cross-sectional design of this study does not permit us to separate the contributions of depression and mania to our structural findings. Although we attempted to control for heterogeneity, there was still variation in levels of medication exposure, comorbidities, and substance exposure, and in time elapsed since the first episode of mania. For example, a lack of white matter or other

regional gray matter differences may have been due to exposure to a variety of neurotrophic agents including lithium, atypical antipsychotics, and antidepressants. The impact of these varying factors on brain morphometry should continue to be examined with a larger sample size of participants categorized based on these specific variables. Furthemore, an integration of brain imaging with longitudinal clinical assessments would aid in determining the impact of burden of illness on brain structure and function and the role of intervention in reversing early volume loss in the sACC.

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