Limbic and Callosal White Matter Changes in Euthymic Bipolar I Disorder: An Advanced Diffusion Magnetic Resonance Imaging Tractography Study

Louise Emsell, Alexander Leemans, Camilla Langan, Wim Van Hecke, Gareth J. Barker, Peter McCarthy, Ben Jeurissen, Jan Sijbers, Stefan Sunaert, Dara M. Cannon, and Colm McDonald

**Background:** White matter microstructural changes detected using diffusion tensor imaging have been reported in bipolar disorder. However, findings are heterogeneous, which may be related to the use of analysis techniques that cannot adequately model crossing fibers in the brain. We therefore sought to identify altered diffusion anisotropy and diffusivity changes using an improved high angular resolution fiber-tracking technique.

**Methods:** Diffusion magnetic resonance imaging data was obtained from 35 prospectively confirmed euthymic bipolar disorder type 1 patients (age 22–59) and 43 control subjects (age 22–59) drawn from a sample of 120 age- and gender-matched demographically similar case-control pairs. Tractography using a constrained spherical deconvolution approach to account for crossing fibers was implemented. Changes in fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity between patient and control groups in subdivisions of the corpus callosum, cingulum, and fornix were measured as indicators of trait differences in white matter microstructural organization in bipolar disorder.

**Results:** Patients had significantly reduced fractional anisotropy and increased mean diffusivity and radial diffusivity in all divisions of the corpus callosum, left fornix, and subgenual cingulum compared with control subjects. Axial diffusivity was increased in the fornix bilaterally and right dorsal-anterior cingulum.

**Conclusions:** By using an improved fiber-tracking method in a clinically homogeneous population, we were able to localize trait diffusivity changes to specific subdivisions of limbic fiber pathways, including the fornix. Our findings extend previous reports of altered limbic system microstructural disorganization as a trait feature of bipolar disorder.

**Key Words:** Bipolar disorder, diffusion imaging, euthymic, fractional anisotropy, MRI, tractography

White matter changes detected by magnetic resonance imaging (MRI) based methods are increasingly being reported in the bipolar disorder (BD) literature (1,2). However, no consensus exists as to the true extent, location, or even direction of these changes. Studies have reported a range of findings encompassing widespread reductions (3–5) to focal increases (6,7) in diffusion anisotropy; global and regional volumetric reduction have also been reported by some groups (8–10), while others report no change in remitted patients (11,12). It is likely that the source of these discrepancies arises from both clinical heterogeneity within the populations studied and the imaging methodology employed, with further variability from small sample sizes and cross-sectional study designs.

Notably, the vast majority of studies reporting white matter microstructural change use diffusion tensor imaging (DTI). This technique aims to relate the random dispersion of water molecules within tissue over a time period of several milliseconds to the underlying tissue microstructure by using a simple mathematical tensor model, to provide an estimate of the principal direction of water diffusion within tissue over a time period of several milliseconds. However, the DTI model fails to incorporate information about the random dispersal of water molecules, which are ubiquitous in the brain (15), mean that results derived purely from DTI analysis may be subject to false negatives and positives attributable to the model itself (16). It follows that the conflicting findings in bipolar disorder reported thus far may have arisen, in part, from limitations associated with the DTI model. By using improved diffusion analysis methods to verify differences in these populations, we can both increase confidence in the veracity of previous findings by replicating them and also increase the sensitivity of our analyses in regions of crossing fibers. This was the core aim of the present study. For a comprehensive review of recent advances in diffusion MRI (dMRI) analysis, see Tournier et al. (17).

In a previous DTI voxel-based analysis (VBA) of this same population, we reported decreases in fractional anisotropy and increases in radial diffusivity in clusters of voxels corresponding to the posterior cingulum and corpus callosum (18). However, the extent to which the effect of smoothing and statistical threshold influenced the degree of change and spatial location of these findings is not known. In the present work, we therefore use a different analysis method, constrained spherical deconvolution...
(CSD) tractography, to investigate these regions in a targeted approach based on our a priori findings.

Diffusion tensor imaging based tractography has been used previously to investigate bipolar disorder and changes in DTI parameters have been found in a number of fiber pathways (11,19–21). However, to minimize multiple hypothesis testing, we restricted our analysis to those bundles that emerged both in our VBA analysis and that have been reported as potentially abnormal in the literature. Additionally, we included the fornix, as this bundle has not previously been investigated using targeted tracking in bipolar disorder despite forming an important pathway between the hippocampus and anterior limbic regions and which also forms a core part of the midline limbic system region in which our VBA findings emerged.

Our study improves on previous methods in a number of ways. First, our clinical sample is well defined, relatively large, and homogeneous with respect to illness subtype (bipolar disorder type I); previous history of psychosis; mood state; recent history of alcohol, substance, and recreational drug misuse (none in the previous year); and lack of significant symptomatology at the time of scanning (all euthymic). Second, we employed a dMRI analysis method for tractography that is able to model more than one intravoxel fiber direction: constrained spherical deconvolution (22). A technical description of the technique is beyond the scope of this article (refer to [22,23]). However, briefly, CSD is a nonparametric method for tractography that is able to resolve different anatomical subdivisions of the corpus callosum, to more comprehensively map the cingulum, and to extract the fornix (which is typically challenging for DTI approaches due to partial volume effects arising from ventricular cerebrospinal fluid and crossing fibers in this region).

In summary, here, we present findings from the first CSD tractography investigation of tract alterations in key limbic white matter pathways involved in emotional and cognitive regulation in bipolar 1 disorder.

Methods and Materials

Participants

Participants between 22 and 60 years old were recruited from the local community as part of the Galway Bipolar Disorder Study, a cohort of 60 euthymic bipolar 1 disorder patients and 60 age and gender pairwise-matched control subjects described previously (18,24). A subset of this group was re-recruited for diffusion MRI scans, of which 35 patients and 43 control subjects were scanned successfully. Exclusion criteria included a history of neurological illness, lifetime (comorbid for patients) DSM-IV Axis 1 disorder, personal or family history of psychotic or affective disorder in first- or second-degree relatives (for control subjects), a history of substance and/or alcohol misuse in the past year, learning disability, and current oral steroid use. Further demographic information is presented in Table 1.

Bipolar disorder type I status and the absence of comorbid psychiatric illness and alcohol or substance misuse were confirmed using the DSM-IV Structured Clinical Interview for DSM Disorders (Non-Patient edition for control subjects) (25). Prospectively confirmed euthymia was defined as a score of <6 on both the 19-point Hamilton Rating Scale for Depression and the Young Mania Rating Scale (26) assessed at two different time intervals, one at least 1 month before data collection and again on the day of scanning, as per Thompson et al. (27). Before commencement of the study, ethical approval was obtained from the National University of Ireland Galway and University College Hospital Galway research ethics committees. After complete description of the study to the subjects, written informed consent was obtained.

Imaging

Data Acquisition. Diffusion tensor imaging data were acquired on a 1.5T Siemens Magnetom Symphony MRI scanner

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**Table 1. Demographics of Subjects Included Within Total Study Population**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Gender: Male/Female</td>
<td>20/15</td>
<td>22/21</td>
</tr>
<tr>
<td>Mean Age in Years (SD)</td>
<td>44 (±10)</td>
<td>42 (±10)</td>
</tr>
<tr>
<td>Age Range in Years</td>
<td>22 to 60</td>
<td>23 to 58</td>
</tr>
<tr>
<td>GAF Score (SD)</td>
<td>85 (±3)</td>
<td>96 (±6)*</td>
</tr>
<tr>
<td>Mean Total Brain Volume in mL (SD)</td>
<td>1491 (±160)</td>
<td>1455 (±152)</td>
</tr>
<tr>
<td>Mean Age of Onset (SD)</td>
<td>29 (±6)</td>
<td>—</td>
</tr>
<tr>
<td>Mean Illness Duration in Years (SD)</td>
<td>12 (±9)</td>
<td>—</td>
</tr>
<tr>
<td>Current Lithium User (n)</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>Current Antipsychotic User (n)</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>Current Anticonvulsant User (n)</td>
<td>18</td>
<td>—</td>
</tr>
<tr>
<td>Current Antidepressant User (n)</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Mean Number of Years of Lithium Therapy (SD)</td>
<td>10 (±7)</td>
<td>—</td>
</tr>
<tr>
<td>Mean Number of Years of Atypical Antipsychotic Therapy</td>
<td>5 (±2)</td>
<td>—</td>
</tr>
<tr>
<td>Lifetime History of Alcohol Abuse (n)</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Reported First-Degree Relative with BD or Schizophrenia (n)</td>
<td>12</td>
<td>—</td>
</tr>
<tr>
<td>Previous Psychotic Episode (n)</td>
<td>32</td>
<td>—</td>
</tr>
<tr>
<td>Mean Number of Hospital Admissions Due to Mania (Range)</td>
<td>3 (0–12)</td>
<td>—</td>
</tr>
<tr>
<td>Mean Number of Hospital Admissions Due to Depression (Range)</td>
<td>1 (0–5)</td>
<td>—</td>
</tr>
</tbody>
</table>

A summary of the core demographics of the study population with regard to age, gender, and key clinical data. BD, bipolar disorder; GAF, Global Assessment of Functioning; SD, standard deviation. *The patient mean and the control mean are significantly different (t = 12.81, p ≤ .001).
(Erlangen, Germany) using an 8-channel head coil with an echo planar image diffusion sequence acquired with parallel imaging (factor 2), 64 optimized diffusion gradient directions (based on [28]), 60 contiguous axial slices, \( b = 1300 \text{ s/mm}^2 \), seven nondiffusion weighted images, repetition time \( = 8100 \text{ milliseconds} \), echo time \( = 95 \text{ msec} \), field of view \( = 240 \times 240 \text{ mm}^2 \), matrix \( = 96 \times 96 \), in-plane voxel size of \( 2.5 \times 2.5 \text{ mm}^2 \), slice thickness \( = 2.5 \text{ mm} \).

**Preprocessing.** The diffusion weighted imaging data quality were checked qualitatively using a suite of quality assurance tools in a freely available dMRI analysis software package ExploreDTI (www.exploredti.com) (29) and corrected for motion and distortions using the same software. During motion correction, the \( b \)-matrix was rotated and the signal intensity modulated to preserve the orientational and quantitative information altered during warping (30,31).

**Construction of a Population Atlas.** All the motion and distortion corrected datasets were subsequently coregistered to each other using a nonrigid approach (32), to create a study population atlas (PA) (33,34).

**Whole-Brain Tractography.** Fiber tracking was performed in the PA using a modified deterministic CSD approach (22) implemented in ExploreDTI (35). Tracking was initiated in every voxel and continued with a step size of 1 mm until one of the following thresholds was exceeded: fiber orientation distribution \( >0.15 \), angle \( <45^\circ \), minimum length \( <20 \text{ mm} \), and maximum length \( >300 \text{ mm} \). A conservative spherical harmonic order, \( L_{\text{max}} = 6 \), was used to prevent overfitting due to noise.

**Tract Selection in Atlas Space.** Tract masks were created in the PA using manually defined inclusion, AND, and exclusion, NOT, regions of interest (ROIs). In this approach, an AND ROI selects only fibers traversing the ROI, while a NOT ROI excludes any fiber that passes through it. A single-rater defined the ROIs that were tailored to the PA and based on expert neuroanatomical knowledge of known pathways derived from classical anatomical descriptions (36,37), contemporary dMRI based atlases (38,39), and recent investigations (40). Such an approach has successfully been applied previously to improve tract delineation (41). The following masks were created: corpus callosum; genu, occipital, parietal, and temporal splenium; cingulum; and fornix. To improve the specificity of the analysis, the cingulum and fornix tract masks were parcellated into subregions. The cingulum was divided into subgenual, anterior dorsal, posterior dorsal, and parahippocampal sections. The fornix was divided into left and right segments using an AND ROI ipsilaterally and NOT ROI contralaterally. These masks are illustrated in Figure 1.
Differences in Diffusion Parameters Between BD Patients and Control Subjects in Different Divisions of the Cingulum and Fornix

**Table 2. Results of Whole Tract Assessment**

<table>
<thead>
<tr>
<th>Tract</th>
<th>FA</th>
<th>MD</th>
<th>AD</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cingulum</td>
<td>$F = 2.621$</td>
<td>$F = 1.557$</td>
<td>$F = .033$</td>
<td>$F = 2.433$</td>
</tr>
<tr>
<td></td>
<td>$p = .110$</td>
<td>$p = .216$</td>
<td>$p = .857$</td>
<td>$p = .123$</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>$F = 10.56$</td>
<td>$F = 7.386$</td>
<td>$F = 1.264$</td>
<td>$F = 10.26$</td>
</tr>
<tr>
<td></td>
<td>$p = .002^a$</td>
<td>$p = .008^a$</td>
<td>$p = .265$</td>
<td>$p = .002^a$</td>
</tr>
<tr>
<td>Fornix</td>
<td>$F = 3.849$</td>
<td>$F = 5.442$</td>
<td>$F = 4.572$</td>
<td>$F = 5.588$</td>
</tr>
<tr>
<td></td>
<td>$p = .054$</td>
<td>$p = .022^a$</td>
<td>$p = .036^a$</td>
<td>$p = .021^a$</td>
</tr>
</tbody>
</table>

Results of the repeated-measures analysis of covariance analyses combining all the tract divisions of each tract into three single bundles. In these models, hemisphere and tract division were within-subject variables, diagnosis and gender were between-subject fixed factors, and age and total brain volume were covariates.

AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

$^a$Statistically significant results, $p < .05$, following Bonferroni correction.

**Diffusion Parameter Extraction.** The tract masks were applied to each individual PA registered dataset and values of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were extracted from all the voxels included in each mask (41). These masks were then modulated by the track density of each bundle to weight the diffusion metrics toward those voxels most likely to contain reliable portions of the tract in every individual. The diffusion parameters are derived from the diffusion tensor and each characterize a different aspect of diffusivity (42). The FA describes the degree of anisotropy within a voxel and may be related to the orientation and geometric properties of axons within the voxel. The mean diffusivity describes the average amount of diffusion within a voxel and can be related to intracellular and extracellular water content. The axial and radial diffusivities relate to the amount of diffusion along and perpendicular to the principal direction of diffusion, respectively. They may be related to axonal number and degree of myelination. In practice, however, all the parameters also reflect nonbiological features of the image and their interpretation is not trivial (43–45).

**Statistical Analysis.** A global assessment of each tract was obtained using repeated measures analysis of covariance models, with hemisphere and tract division as within-subject variables, diagnosis and gender as between-subject fixed factors, and age and total brain volume as covariates. The dependent variables were the diffusion parameters FA, MD, AD, and RD. Post hoc multivariate analyses of covariance with the same between-subject factors were used to investigate differences in the tract segments between patients and control subjects. Bonferroni correction for multiple comparisons was applied post hoc using standard calculations in IBM SPSS Statistics version 19 (IBM Corp, Armonk, New York). Additionally, partial correlations correcting for age and total brain volume were performed for several key clinical variables of interest selected a priori. These variables were illness duration in years, lithium therapy duration in years, antipsychotic therapy in years, and previous maximum weekly consumption of alcohol in units. As patient-reported previous alcohol consumption may not be sufficiently reliable and represent periods of binge drinking, an additional multivariate analysis of covariance was performed including lifetime history of alcohol abuse as a factor of interest. Statistical significance was set to $p = .05$.

**Results**

The study population demographics and clinical profile are summarized in Table 1. All patients were medicated at the time of scanning, predominantly with lithium. One third of the sample had a first-degree relative with bipolar disorder or schizophrenia and almost all patients had experienced psychotic symptoms during episodes of illness exacerbation.

**Global Differences in Tract Diffusivity Measures**

We detected significant differences between patients and control subjects in FA, MD, and RD in the corpus callosum. In the fornix, significant differences were found in MD, AD, and RD. In all these cases, anisotropy was decreased and diffusivity increased in patients compared with control subjects. There were no significant group differences in the cingulum. The complete results, including statistical significance, are summarized in Table 2.

**Regional Differences in Tract Diffusivity Measures**

**Fornix.** Significant group differences reflecting increases in all measures of diffusivity (i.e., MD, AD, RD) were detected in both left and right fornix divisions in patients compared with control subjects. Fractional anisotropy was significantly reduced in patients in the left fornix (Table 3).

**Cingulum.** When the divisions of the cingulum were considered separately, significant group differences in FA, MD, and AD, $p < .05$, following Bonferroni correction.

**Table 3. Differences in Diffusion Parameters Between BD Patients and Control Subjects in Different Divisions of the Cingulum and Fornix**

<table>
<thead>
<tr>
<th>Tract</th>
<th>Segment</th>
<th>Left FA</th>
<th>Left MD</th>
<th>Left AD</th>
<th>Left RD</th>
<th>Right FA</th>
<th>Right MD</th>
<th>Right AD</th>
<th>Right RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cingulum</td>
<td>Subgenual</td>
<td>$F = 5.964$</td>
<td>$F = 6.508$</td>
<td>$F = .714$</td>
<td>$F = 8.736$</td>
<td>$F = .000$</td>
<td>$F = .065$</td>
<td>$F = .002$</td>
<td>$F = .960$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = .017^a$</td>
<td>$p = .013^a$</td>
<td>$p = .401$</td>
<td>$p = .004^a$</td>
<td>$p = .996$</td>
<td>$p = .800$</td>
<td>$p = .967$</td>
<td>$p = .758$</td>
</tr>
<tr>
<td>Dorsal-Anterior</td>
<td></td>
<td>$F = 1.019$</td>
<td>$F = .793$</td>
<td>$F = .024$</td>
<td>$F = 1.522$</td>
<td>$F = .6126$</td>
<td>$F = .001$</td>
<td>$F = 5.161$</td>
<td>$F = 1.419$</td>
</tr>
<tr>
<td>Dorsal-Posterior</td>
<td></td>
<td>$F = 1.207$</td>
<td>$F = 1.389$</td>
<td>$F = .289$</td>
<td>$F = 1.552$</td>
<td>$F = .825$</td>
<td>$F = .540$</td>
<td>$F = .001$</td>
<td>$F = .964$</td>
</tr>
<tr>
<td>Parahippocampus</td>
<td></td>
<td>$F = .49$</td>
<td>$F = 1.981$</td>
<td>$F = 1.572$</td>
<td>$F = 1.682$</td>
<td>$F = .425$</td>
<td>$F = 1.795$</td>
<td>$F = 1.127$</td>
<td>$F = 1.874$</td>
</tr>
<tr>
<td>Fornix</td>
<td></td>
<td>$F = 3.999$</td>
<td>$F = 5.600$</td>
<td>$F = 4.517$</td>
<td>$F = 5.784$</td>
<td>$F = 3.135$</td>
<td>$F = 5.018$</td>
<td>$F = 4.241$</td>
<td>$F = 5.134$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = .049^a$</td>
<td>$p = .021^a$</td>
<td>$p = .033^a$</td>
<td>$p = .019^a$</td>
<td>$p = .081$</td>
<td>$p = .028^a$</td>
<td>$p = .043^a$</td>
<td>$p = .026^a$</td>
</tr>
</tbody>
</table>

Results of the multivariate analysis of covariance analysis of cingulum and fornix subdivisions. In this model, diagnosis and gender were between-subject fixed factors and age and total brain volume were covariates.

AD, axial diffusivity; BD, bipolar disorder; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

$^a$Statistically significant results, $p < .05$, following Bonferroni correction.
Table 4. Differences in Diffusion Parameters Between BD Patients and Control Subjects in Different Divisions of the Corpus Callosum

<table>
<thead>
<tr>
<th>Segment</th>
<th>FA</th>
<th>MD</th>
<th>AD</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu</td>
<td>$F = 8.195$</td>
<td>$F = 3.406$</td>
<td>$F = .049$</td>
<td>$F = 6.073$</td>
</tr>
<tr>
<td></td>
<td>$p = .005^a$</td>
<td>$p = .069$</td>
<td>$p = .825$</td>
<td>$p = .016^b$</td>
</tr>
<tr>
<td>Parietal</td>
<td>$F = 6.075$</td>
<td>$F = 4.826$</td>
<td>$F = 5.91$</td>
<td>$F = 6.572$</td>
</tr>
<tr>
<td></td>
<td>$p = .016^a$</td>
<td>$p = .031^a$</td>
<td>$p = .448$</td>
<td>$p = .012^b$</td>
</tr>
<tr>
<td>Occipital</td>
<td>$F = 6.765$</td>
<td>$F = 5.285$</td>
<td>$F = .625$</td>
<td>$F = 7.566$</td>
</tr>
<tr>
<td></td>
<td>$p = .011^a$</td>
<td>$p = .024^a$</td>
<td>$p = .432$</td>
<td>$p = .007^a$</td>
</tr>
<tr>
<td></td>
<td>$p = .009^a$</td>
<td>$p = .003^a$</td>
<td>$p = .083$</td>
<td>$p = .001^a$</td>
</tr>
</tbody>
</table>

Results of the multivariate analysis of covariance analysis of corpus callosum subdivisions. In this model, diagnosis and gender were between-subject fixed factors, and age and total brain volume were covariates.

*AD, axial diffusivity; BD, bipolar disorder; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

*Statistically significant results, $p < .05$, following Bonferroni correction.

Corpus Callosum. We detected significant differences between patients and control subjects in FA and RD in the callosal genu. In the parietal, occipital, and temporal splenium, we found differences in FA, MD, and RD (see Table 4 for complete results). Again, in all callosal divisions, anisotropy was decreased and diffusivity increased in patients compared with control subjects.

Clinical Correlations

We did not find any significant association between illness duration, duration of lithium and antipsychotic therapy, previous maximum weekly consumption of alcohol in units or lifetime history of alcohol abuse, and any DTI parameter in the tracts in which significant differences between patients and control subjects are reported. Results are available on request.

Discussion

The findings presented provide substantial evidence of altered white matter microarchitectures in key limbic system pathways as a trait feature of bipolar disorder type I. The fornix has not been investigated a priori in bipolar disorder and the result of decreased FA bilaterally is the first tractography finding that demonstrates potential abnormalities in this tract. The findings in the cingulum bundle and corpus callosum are consistent with other studies investigating smaller patient samples with DTI and localize anisotropy and diffusivity changes to specific subdivisions of these bundles.

Fornix

The fornix is the main efferent pathway of the hippocampus and connects many limbic regions, including the septal nuclei, nucleus accumbens, thalamus, and cingulate cortex (37). All of these structures have been implicated to varying degrees in both structural and functional studies in BD (46). However, the fornix itself has not been investigated a priori in BD, possibly due to the aforementioned imaging challenges associated with investigating the bundle. Indeed, even using CSD, partial volume effects prevented us from accurately constructing the fornicical columns, which diverged erroneously into the anterior commissure instead of splitting into precommissural and postcommissural divisions. One case study, however, has proposed compression of the fornix as the cause of bipolarity (47), while a voxel-based DTI study noted FA reduction in this region in familial bipolar adolescents (48). The only recent DTI tractography study of the fornix in psychotic disorder was performed in schizophrenia and reported FA reductions in chronic male patients compared with control subjects (49).

Functionally, as the core efferent fiber system of the hippocampus, the fornix is involved in mnemonic function, which has been shown to be impaired in BD (50,51). Fornical volume reduction has also been implicated in declining cognitive function during the development of Alzheimer’s disease (52). It is conceivable, then, that fornix abnormalities may contribute to the neuropsychological deficits that have been reported in bipolar disorder (53).

Corpus Callosum

We detected diffusion changes in all divisions of the corpus callosum that we investigated. The finding in the genu has been reported previously (54–56) and is consistent with the hypothesis that cognitive and emotional regulation may be disrupted due to compromised interhemispheric information transfer between anterior paralimbic regions such as the dorsal, ventral, and orbitofrontal cortices. Abnormalities in these prefrontal regions have been reported in both functional (57) and structural neuroimaging studies of bipolar disorder (2). Disrupted prefrontal connectivity could give rise to a number of neuropsychological and psychopathological impairments present in BD, including response inhibition, attentional and working memory deficits, and altered response to emotional stimuli such as faces or words (58).

Alterations in the callosal splenium have also been reported previously; however, voxel-based analyses are unable to localize such differences to specific tracts, and DTI-based tractography tends to select fibers contained predominantly within the forceps major. Notably, in a DTI voxel-based analysis of this sample, we also found decreases in FA and increases in RD in clusters of voxels corresponding to the callosal splenium (18). It is generally accepted that the corpus callosum is parcellated into different functional regions that correspond closely with its anatomical projections (40,59,60). To our knowledge, we are the first to investigate differences in subdivisions of the splenium that project to different anatomical regions. We demonstrated changes in all occipital, parietal, and temporal projections of the splenium, suggesting that diffusivity changes may not be specific to the forceps major but diffuse within the entire corpus callosum. We did not investigate the callosal body for a number of methodological reasons. These include the structure falling outside the scope of our initial hypotheses due to its anatomical connections with motor and somatosensory cortical regions; to limit multiple hypothesis testing; and because our exploratory VBA study did not detect any significant alterations in this region. Nevertheless, previous studies have identified volumetric reduction, reduced FA, and increased RD in this region (61–63).

Cingulum

The cingulum is a complex fiber system that forms a central component to the entire limbic network. This makes it an attractive candidate for abnormality in psychiatric illness. Indeed, structural changes in this region have been documented in BD (64,65) and schizophrenia (66). However, although the core dorsal component is readily mapped with DTI, the subgenual and parahippocampal divisions are seldom fully characterized, while cingulo-frontal and parietal projections are typically reconstructed.
to widely varying extents in different subjects, making group comparisons problematic. For these reasons, in a previous feasibility study, we focused only on the core dorsal segment and did not find any differences (67). However, by using CSD in combination with the PA approach, we were able to investigate the subgenual and parahippocampal divisions, as well as divide the dorsal segment into more functionally sensible anterior and posterior regions. When assessing the whole tract, we found no differences, as in the previous analysis; however, when investigating the subdivisions separately, we identified significant FA reductions in the subgenual and anterior segments consistent with previous reports by other groups (64). It is, therefore, possible that previous negative findings were the result of a lack of sensitivity to detect subtle regional changes in this bundle.

Methodological Considerations

There are a number of methodological issues, both clinical and technical, that should be highlighted.

Although the clinical population was homogeneous with respect to bipolar disorder type I subclass, illness severity, and mood state, as in all studies of this type, there remained differences among the patients with respect to the relative number of depressive or manic episodes, illness duration, medication status, family history of psychotic illness, and previous lifetime history of alcohol abuse.

The cross-sectional design, patient sample size, and multiple comparison problems associated with performing many correlations on the many possible variables of interest preclude us from drawing statistically sound conclusions about smaller subgroups. Nevertheless, we performed tentative partial correlations to investigate associations between illness duration, lifetime alcohol abuse, lithium and atypical antipsychotic therapy, and diffusion parameters and found no statistically significant associations. For the reasons highlighted above, it is possible that we lacked the power to detect such correlations. Alterations in white matter have been associated with some of these factors in previous studies. For example, in a recent review, Schneider et al. (68) described evidence for neuroprogressive changes in bipolar disorder, including DTI studies that have reported an effect of illness duration on FA (7). However, they concluded that, in general, evidence of neuroprogressive white matter change is sparse and its interpretation complicated by the interplay of many other factors such as limited prospective data, age effects, and clinical heterogeneity.

The effect of medication on diffusion measures is impossible to elucidate statistically in small-scale cross-sectional bipolar disorder neuroimaging studies, where heterogeneous polypharmacy prevails, and this is also a limitation present in our study. Although the evidence of lithium-related changes in structural MRI studies of gray matter have been reported with some degree of consistency, associations with lithium therapy and other medications and DTI changes are far less widely reported (69). Where medication effects have been reported in longitudinal neuroimaging studies, they tend to be normalizing with respect to healthy control subjects and consequently such effects may be even more difficult to detect in typical small cross-sectional imaging studies (69).

Previous alcoholism has been associated with changes in FA and MD (70). Given the well-documented comorbidity that exists between harmful use of alcohol and bipolar disorder, alcohol lifetime use may, therefore, also be a potent and problematic confounder in the study of bipolar disorder patients (71).

Recruitment of a sample of BD patients without such comorbidity, however, represents a significant challenge. Furthermore, accurately recording the prevalence of lifetime harmful alcohol use requires accurate recall by patients of previous alcohol consumption, thus introducing the possibility of recall bias or under-reporting due to stigma. In our cohort, 7 out of 35 BD patients reported a lifetime history of harmful use of alcohol, confirmed by examination of case notes. We also recorded self-reported current and previous maximum number of units of alcohol consumed per week. In both our analyses investigating the effect of previous alcohol abuse on DTI parameters, we found no significant effects, suggesting that in this sample, the effect of previous alcohol abuse does not contribute significantly to our findings.

A key aim of this work was to identify traits microstructural changes in a specific BD subpopulation; therefore, the results may not extend to the entire spectrum of the disorder. Notably, the majority of our patient group had previously experienced at least one episode of psychosis and a third had at least one first-degree relative diagnosed with a psychotic or affective disorder. Diffusion tensor imaging metric alterations, particularly in FA, have been associated with psychotic features (72,73) and have been associated with genetic risk (74,75). Indeed, white matter alterations have been proposed as a potential bipolar endophenotype (56,74). It is, therefore, possible that the FA and diffusivity changes we detected could be driven by these elements of our patient group, which may represent white matter changes associated with a more severe end of the bipolar spectrum.

With regard to technical considerations, as with all tractography studies, there are limitations that arise from the challenges of modeling complex microarchitecture based on diffusion within voxels 2.5mm$^3$ in size. Although the CSD tract masks more closely resemble known anatomy than DTI tractography allows, CSD is still subject to issues associated with parameter selection similar to those for DTI-based methods (e.g., fiber orientation distribution, angle/curvature, length thresholds, etc.) and CSD results do not represent the anatomical ground truth. Furthermore, although we were fully able to exploit the high angular resolution of our data by using CSD analysis for tractography, in the absence of any other presently accepted alternative measure, we used DTI-derived indices in our statistical analysis. These metrics suffer from the same limitations of the DTI model as described previously and cannot be used to infer anything specific about how the microstructure is affected, such as whether there is more or less myelin or higher or lower axonal density (76). Nevertheless, there is some evidence from genetic association studies and postmortem findings reporting down-regulation of oligodendrocyte- and myelin-related genes and reductions in oligodendrocytes to support changes in myelination in bipolar disorder (77–79). The development of novel MRI acquisition schemes, such as myelin water imaging (80) and axonal density mapping (81) in combination with metrics that could provide more useful quantitative information, is presently a focus of investigation in the field (82,83). Finally, a core part of our analysis involved extracting data from images coregistered to a population atlas. The assumption that each voxel within a mask corresponds to precisely the same location in each tract in each subject is compromised by issues of image registration and individual differences in anatomy (84).

Conclusion

In conclusion, we have used an advanced tractography analysis that improves upon previous DTI methods and have identified
diffusivity changes in bipolar disorder in key white matter tracts including the corpus callosum, cingulum bundle, and fornix. Taken together, the results of this investigation both support and extend previous evidence that trait microstructural changes may compromise limbic networks in bipolar disorder patients.

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