Fixel-Based Diffusion Magnetic Resonance Imaging Reveals Novel Associations Between White Matter Microstructure and Childhood Aggressive Behavior

Rachael Grazioplene, Wan-Ling Tseng, Kimberly Cimino, Carla Kalvin, Karim Ibrahim, Kevin A. Pelphrey, and Denis G. Sukhodolsky

ABSTRACT

BACKGROUND: Childhood aggression has been linked to white matter abnormalities, but research has been inconsistent with regard to both regions of alterations and directionality of the associations. We examined white matter microstructure correlates of aggression using a novel diffusion imaging analysis technique, fixel-based analysis, which leverages connectivity and crossing-fiber information to assess fiber bundle density.

METHODS: The sample included 70 children with aggressive behavior and 25 healthy control children without aggressive behavior. Aggression was measured by the parent-rated Aggressive Behavior scale of the Child Behavior Checklist. Fixel-based analysis was conducted at the whole-brain and region-of-interest levels, including the uncinate fasciculus, inferior longitudinal fasciculus, fornix, cingulum bundle, and genu, body, isthmus, and splenium of the corpus callosum.

RESULTS: Whole-brain analysis of covariance revealed that children with aggressive behavior, relative to control children, had lower fiber density in a cluster of limbic and cortical pathways, including the inferior fronto-occipital fasciculus, fornix, middle cerebellar peduncle, and superior thalamic radiations (familywise error-corrected $p < .01$), and had higher fiber density in the corpus callosum (body and splenium) (familywise error-corrected $p < .05$). Region-of-interest analyses showed decreased fiber density in cingulum bundles associated with aggression. These effects were independent of age, sex, IQ, symptoms of attention-deficit/hyperactivity disorder, medications, and head motion. In children with aggressive behavior, co-occurring callous-unemotional traits and anxiety did not moderate the association between aggression and white matter density.

CONCLUSIONS: Diminished white matter density in pathways connecting limbic and cortical regions is associated with childhood aggression. Abnormal interhemispheric connectivity via corpus callosum may also reflect a potential neural mechanism involved in aggression.

Keywords: Aggression, Callous-unemotional traits, Diffusion-weighted imaging, Disruptive behavior disorders, Fixel-based analysis, White matter microstructure

https://doi.org/10.1016/j.bpsc.2019.12.018

Aggressive behavior is common in many psychiatric disorders with childhood onset, including conduct disorder, oppositional defiant disorder, and disruptive mood dysregulation disorder (1–3). It is among the most frequent reasons for referral to mental health services (3,4). Aggression in childhood is associated with a host of adverse outcomes later in life, including alcohol and drug abuse, violent crimes, depression, suicide attempts, and unemployment (5–7). The aggression-related health and economic burden on the perpetrators and victims highlights a critical need to understand the etiology and pathophysiology of this debilitating phenotype.

Emerging evidence from neuroimaging research suggests that aggression is mediated by abnormalities in brain networks critical to emotion regulation, affective processing, and reward learning (8–10). For example, heightened reactivity in the amygdala, coupled with insufficient prefrontal regulation, can result in aggressive responses to frustration or provocation (9). One way to investigate abnormalities in the neural circuits associated with aggression is via diffusion-weighted imaging (DWI), a magnetic resonance imaging (MRI) technique for measuring structural connectivity between brain regions. DWI captures information about the structure of bundled axon populations by measuring the diffusion of water molecules within and along the white matter pathway (11). This imaging technique can be used to examine anatomical disruptions or alterations in white matter tracts that are associated with aggression.
One commonly used metric to assess white matter integrity is fractional anisotropy (FA), which reflects white matter microstructure properties such as axon packing density and degree of myelination (12,13). Studies of aggression in adults have yielded a more consistent picture of aggression-linked white matter abnormalities compared with the child literature, which consists of more heterogeneous samples with a mix of clinic-referred, high-risk, and community samples and a wider age range (14). Nonetheless, existing evidence suggests that aggression in childhood is linked to FA in major association fibers such as the uncinate fasciculus (15–20), cingulum (18,19,21,22), superior longitudinal fasciculus (19,22–24), and inferior fronto-occipital fasciculus (18,22,25); commissural fibers such as the corpus callosum (19,22–24,26); and projection fibers such as the anterior corona radiata (18,19,22,26). Although the majority of past studies reported decreased FA associated with aggression, a few studies reported increased FA associated with aggression. This discrepancy may be partly due to the interpretation of FA, in the absence of other diffusion tensor imaging metrics, in brain regions containing crossing fibers (27,28).

To address this issue, we used fixed-based analysis (29,30), which is better suited than the voxel-based approach to provide anatomically specific information about the micro- and macrostructural properties of white matter populations, particularly in crossing fiber regions. While FA captures information about overall relative diffusion patterns in a voxel, studies in both phantom and human data show that fixed-based analysis can approximate both the density and macrostructural cross section of distinct fiber bundles traversing a voxel (29,30). The primary measure of white matter microstructure used in this study is fiber density (FD) derived from the fixed-based analysis. It is a microscopic estimate of the density of axons within a particular fiber population in a given voxel. The primary aim of this study was to examine the association of childhood aggression with regional white matter microstructure as indexed by FD. Although FA and FD are positively correlated (31), FD (derived from fixed-based analysis) is capable of capturing information about multiple fiber pathways that cross through voxels. Compared with FA, FD is less prone to diffusion measurement confounds related to crossing fibers and can provide more anatomically accurate information about pathway-specific diffusion alterations (32). Because up to 90% of voxels in a DWI analysis contain crossing fibers (33), the use of FD instead of FA offers greater anatomical specificity (see Supplement for details).

The investigation of neural mechanisms of aggressive behavior also needs to consider possible effects of co-occurring forms of psychopathology. Specifically, callous-unemotional (CU) traits, defined by a lack of guilt, empathy, or remorse, often co-occur with aggressive and antisocial behaviors (34). Similarly, anxiety symptoms are often reported in children with clinically significant aggression (particularly reactive impulsive aggression) despite the seemingly counterintuitive link between the two syndromes (35). However, the effects of co-occurring CU traits and anxiety symptoms on white matter microstructure associated with aggression are poorly understood and may have partly contributed to the inconsistent literature. Indeed, recent studies reveal differences in white matter microstructure between adolescents with conduct disorder with high levels of CU traits and those with low levels (17,36). No research so far has investigated the moderating effect of anxiety on the association between white matter and aggression, although evidence suggests that limbic structures such as the amygdala may be differentially involved in aggressive behavior stemming from anxiety (e.g., exaggerated response to affective stimuli) versus CU traits (e.g., reduced response to social cues of distress) (37). Thus, the second aim of this study was to examine the moderating effects of CU traits and anxiety on white matter microstructure of aggression.

This is the first study to use fixed-based analysis to characterize FD in a large and well-characterized sample of children with aggressive behavior relative to a sample of healthy control children without a history of aggressive behavior. First, we conducted whole-brain analysis of covariance to examine FD differences in children with aggressive behavior versus healthy control children with sex, age, IQ, and head motion as covariates. We also conducted whole-brain regression in the group of children with aggressive behavior to examine the association between FD and severity of aggression and to test for interaction effects of aggression by CU traits and aggression by anxiety. The advanced diffusion algorithms and registration capabilities of MRtrix3’s whole-brain fixed analysis pipeline make it possible to examine brain–trait links at an unprecedented level of anatomical specificity, which could lead to novel insights into the pathophysiology of severe mental illness in childhood. In addition to the whole-brain analyses, we examined group differences and symptom correlations with FD in 12 a priori regions of interest (ROIs): left and right uncinate fasciculi, left and right inferior longitudinal fasciculus, left and right fornix, left and right cingulum bundle, and four subdivisions of the corpus callosum (genu, body, isthmus, and splenium). These regions are among the most consistently reported in previous studies (15–20,22–24,38,39) despite the inconsistent directionality of the effects (both lower FA and higher FA were associated with aggression). Given that a larger number of studies reported association of decreased FA with aggression, we hypothesized that children with aggressive behavior, relative to healthy control children, would have lower FD in regions reported in previous studies (i.e., uncinate fasciculus, corpus callosum, cingulum, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, and anterior corona radiata). Moreover, we hypothesized that among children with aggressive behavior, higher levels of aggression would be associated with lower FD in these regions. We also hypothesized that CU traits and anxiety would moderate the relationships between aggression and FD in these regions; however, we did not make specific hypotheses regarding the post hoc patterns of these interactions given the scarce literature in these areas.

METHODS AND MATERIALS
Sample Recruitment and Clinical Characteristics
This was a cross-sectional study that included two groups of children aged 8 to 16 years: 70 children with aggressive behavior and 25 typically developing healthy control children matched for age and sex. The majority of children with aggressive behavior (72.9%) met criteria for oppositional
Table 1. Sample Characteristics of Total Sample (N = 95)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Control Children (n = 25)</th>
<th>Children With Aggressive Behavior (n = 70)</th>
<th>Group Difference</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Years</td>
<td>12.6 (1.6)</td>
<td>11.7 (2.2)</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Full-Scale IQ</td>
<td>111.2 (12.1)</td>
<td>108.2 (12.5)</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>CBCL Aggressive Behavior T Score</td>
<td>50.7 (1.9)</td>
<td>75.9 (7.2)</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>MASC Anxiety Total T Score</td>
<td>43.3 (4.3)</td>
<td>55.8 (12.9)</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>ICU Total Score</td>
<td>15.1 (6.4)</td>
<td>33.8 (9.4)</td>
<td>&lt; .001</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (48)</td>
<td>49 (70)</td>
<td>.06</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>.39</td>
<td></td>
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<tr>
<td>White</td>
<td>17 (68)</td>
<td>56 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>5 (20)</td>
<td>8 (11.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
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<td>1 (1.4)</td>
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<td></td>
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<tr>
<td>American Indian/Alaska</td>
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<td>2 (2.9)</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Other/More than one race</td>
<td>3 (12)</td>
<td>3 (4.3)</td>
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<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (12)</td>
<td>14 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>22 (88)</td>
<td>56 (80)</td>
<td></td>
<td></td>
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<td>DSM-5 Diagnosis</td>
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<td></td>
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<td></td>
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<tr>
<td>Oppositional defiant disorder</td>
<td>51 (72.9)</td>
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<tr>
<td>Conduct disorder</td>
<td>10 (14.3)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Disruptive behavior disorder NOS</td>
<td>2 (2.9)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DMDD</td>
<td>16 (22.9)</td>
<td></td>
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<td></td>
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<tr>
<td>ADHD</td>
<td>54 (77.1)</td>
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<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>18 (25.7)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Depressive disorder</td>
<td>4 (5.7)</td>
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<tr>
<td>Medication</td>
<td>31 (44.3)</td>
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<tr>
<td>Type of Medication</td>
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<td></td>
<td></td>
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<tr>
<td>Stimulants</td>
<td>24 (34.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonstimulants</td>
<td>14 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>6 (8.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroleptics</td>
<td>8 (11.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood stabilizers</td>
<td>1 (1.4)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

ADHD, attention-deficit/hyperactivity disorder; CBCL, Child Behavior Checklist; DMDD, disruptive mood dysregulation disorder; ICU, Inventory of Callous-Unemotional Traits; MASC, Multidimensional Anxiety Scale for Children; NOS, not otherwise specified.

Group differences were examined using the chi-square test for categorical variables and independent-samples t test for continuous variables.

In total, 81.4% met criteria for two or more psychiatric disorders.

Following DSM-5, oppositional defiant disorder diagnosis was not assigned to children who met criteria for DMDD.

defiant disorder, 22.9% met criteria for disruptive mood dysregulation disorder, and 14.3% met criteria for conduct disorder. Demographic and clinical characteristics of the study groups and the whole sample (N = 95) are presented in Table 1. Correlations among variables of interest for the group of children with aggressive behavior are presented in Supplemental Table S1. Children with aggressive behavior participated in a clinical trial of behavior therapy, and this study combines their baseline data with the data of healthy control children. The healthy control children were recruited from the community via advertisements, flyers, and brochures. Each participant’s parent provided informed consent according to specifications by the institutional review board at the Yale University School of Medicine. Each child provided assent.

Children with aggressive behavior were required to meet a cutoff criterion of a T score ≥ 65 on the Aggressive Behavior scale of the parent-rated Child Behavior Checklist, a well-established measure of child psychopathology (40). A T score ≥ 65 is 1.5 SD units above the mean in the standardization sample and represents a cutoff for a clinically significant level of aggression. The Aggressive Behavior scale consists of 18 items reflecting inappropriate anger outbursts as well as verbal and physical aggression. Healthy control children had no current or past history of psychiatric disorders and had a T score < 55 on the Aggressive Behavior scale of the Child Behavior Checklist. All children received a comprehensive diagnostic evaluation that included the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version (41), which is a structured interview with excellent reliability that was conducted with parent and child by an expert clinician to establish DSM-5 diagnoses. Full-scale IQ was evaluated with the Wechsler Abbreviated Scale of Intelligence (42). CU traits were assessed using the parent-rated Inventory of Callous-Unemotional Traits (43), a 24-item questionnaire with excellent reliability and validity (44) (Cronbach’s alpha = .90 in this study). The Multidimensional Anxiety Scale for Children–Second Edition (45) and the Swanson, Nolan, and Pelham–Version IV attention-deficit/hyperactivity disorder (ADHD) scale (46) were completed by parents to evaluate severity of anxiety and ADHD symptoms.

Neuroimaging Data Acquisition and Preprocessing

MR imaging was acquired using a 3T Magnetom Tim Trio system (Siemens Corp., Erlangen, Germany). Diffusion-weighted data were collected with a 32-channel head coil using parallel imaging to gain better signal intensity at air-tissue interfaces. (Diffusion imaging parameters are reported in the Supplement.)

Fixel-Based Processing and Analysis

All participants’ data were subjected to visual inspection by an expert (RG) prior to inclusion in the analysis pipeline; no participants were excluded based on the presence of visually apparent MR artifacts. All fixel-based processing steps were conducted using the MRtrix3 software suite (version RC3) according to the procedures outlined in the MRtrix3 documentation (https://MRtrix.readthedocs.io/en/latest/) (30). Following dcm2niiv conversion, data were preprocessed according to the MRtrix3 fixel-based workflow. All images were denoised (47), eddy-induced distortions corrected (via FSL’s EDDY), and then bias field corrected. Based on the head motion data generated by EDDY, scans with the lowest amount of head movement per participant were selected for subsequent inclusion. One participant was excluded on the
basis of mean framewise displacement (mean displacement > 2 mm for all three DWI acquisitions).

Within MRtrix3’s software capabilities, $b = 0$ images can be leveraged as a second shell from which cerebrospinal fluid (CSF)-specific response functions are estimated in each participant. This calculation allows for multi-shell, multi-tissue deconvolution using single-shell data, thereby enhancing the signal from white matter relative to CSF (48). Per-subject tissue-specific response functions were computed and then averaged to create sample-specific response functions for white matter and CSF (48).

Constrained spherical deconvolution was performed for white matter and CSF using the previously estimated response functions. White matter fiber orientation distribution (FOD) images were then coregistered to create a sample-specific FOD template. Peak FOD amplitudes at each pixel in this template were calculated and thresholded (at 0.30) to remove pixels containing residual gray matter peaks. This thresholded image was then used to create an analysis pixel mask and an analysis voxel mask, which represent the coregistered fixed space and voxel space in white matter across all subjects in the sample. Individual subjects’ FOD images were then transformed into template space, segmented into FD-based pixels within each voxel, and reoriented to the template. Final per-subject FD images were computed by taking each pixel in reoriented subject space and assigning it to the corresponding pixel in template space.

**Whole-Brain Analyses**

Whole-brain tractography was performed using the sample-specific FOD template, and the resulting tractogram of 20 million streamlines was reduced using spherical deconvolution informed filtering of tractograms (49) to contain 2 million streamlines. Pixel-based analyses using connectivity-based fixed pixel enhancement were performed with the fixelfcstats command. This technique employs the generalized linear model with nonparametric permutation testing (5000 permutations) and performs threshold-free cluster enhancement based on underlying tractography.

We conducted whole-brain analysis of covariance comparing children with aggressive behavior ($n = 70$) versus healthy control children ($n = 25$) while covarying for sex, age, IQ, and mean relative subject head motion. We also conducted whole-brain regression in the group of children with aggressive behavior to examine the association between FD and symptom severity of aggression and to test for interaction effects of aggression by CU traits and aggression by anxiety with sex, age, IQ, and head motion included as covariates. All reported significance values are familywise error (FWE) corrected. Extracted FD values were analyzed using R statistical software (50).

**ROI-Based Analyses**

Based on the literature, we selected 12 ROIs (left and right uncinate fasciculus, left and right inferior longitudinal fasciculus, left and right fornix, left and right cingulum bundle, and genu, body, isthmus, and splenium of the corpus callosum) (Figure 1). These ROIs were generated by an expert (RG) based on published tractography/histological atlases (51,52). The corpus callosum was segmented into four subregions (genu, body, isthmus, and splenium) because they each connect to different regions of the brain and also because previous studies implicated aggression-related white matter abnormalities in different subregions of the corpus callosum (19,22–24,26,39). Tracts were generated from the FOD group template using MRtrix3’s tckgen command, based on hand-drawn ROIs and using probabilistic tractography. Mean fiber FD in each region was extracted for each participant and stored for external analysis in R (50). Because we ran 12 statistical tests for each trait/group-based model of interest, we penalized $p$ values using false discovery rate (FDR) correction. We first examined the effect of group membership on FD in each ROI, covarying for sex, age, IQ, and head motion. We then conducted multiple regression analyses in the group of children with aggressive behavior to test for associations between ROI-based FD and aggression scores while controlling for covariates (i.e., sex, age, IQ, and head motion). We also tested interactions of aggression and anxiety as well as aggression and CU (in separate models) to examine whether any associations between FD and aggression differed as a function of these symptoms/traits.

**RESULTS**

**Whole-Brain Analyses**

Taking a categorical approach, whole-brain analysis of covariance in children with aggressive behavior ($n = 70$) versus healthy control children ($n = 25$) revealed that relative to control children, children with aggressive behavior had lower FD in a cluster of white matter fibers within several ventromedial white matter regions in the right hemisphere (FWE-corrected $p < .01$) (Figure 2A, B). Probabilistic tractography analysis revealed that the significant clusters represent at least 5 distinct white matter tracts in the right hemisphere (Figure 2C): the inferior fronto-occipital fasciculus, fornix, middle cerebellar peduncle, and superior thalamic radiations. These results were independent of the effects of sex, age, IQ, and head motion.

Taking a dimensional approach, whole-brain regression analysis in the group of children with aggressive behavior ($n = 70$) revealed a positive association between aggression scores and FD in the body and splenium of the corpus callosum (FWE-corrected $p < .05$) (Figure 3) after covarying for sex, age, IQ, and head motion. Anxiety and CU traits did not show incremental significant associations with FD when included as covariates in the whole-brain model with aggression (FD and anxiety: minimum FWE-corrected $p = .22$; FD and CU traits: minimum FWE-corrected $p = .19$). There was a trend-level significant interaction between aggression and CU traits in the anterior isthmus of the corpus callosum (FWE-corrected $p = .07$) and a trend-level interaction between aggression and anxiety in the posterior body of the corpus callosum (FWE-corrected $p = .06$). Supplemental Figure S1 displays anatomical locations and scatterplots depicting both the CU-aggression and anxiety-aggression interaction effects.

**ROI-Based Analyses**

Taking a categorical approach, we found that children with aggressive behavior had significantly lower FD in the left
cingulum bundle after correcting for multiple comparisons ($B = 0.70$, FDR-corrected $p = .03$) (see Figure 4); this trend was also evident in the right cingulum bundle ($B = 0.63$, FDR-corrected $p = .05$). There were no significant group differences in FD in any of the remaining 10 a priori white matter ROIs after adjusting for multiple comparisons (FDR-corrected $p$ values ranged from .11 to .75) and covarying for sex, age, IQ, and head motion.

Taking a dimensional approach in the group of children with aggressive behavior, there was a significant positive association between aggression and FD in both the corpus callosum body ($B = 0.32$, FDR-corrected $p = .04$) and the corpus callosum isthmus ($B = 0.34$, FDR-corrected $p = .04$) after controlling for sex, age, IQ, and head motion. These ROI-based results mirror the whole-brain finding of a positive association between aggression and FD in callosal fibers. When CU traits and anxiety were included in the ROI models as covariates, there was no evidence that either trait contributed significantly to ROI-based variation in FD in any of the 12 ROIs. Tests of an interaction between anxiety and aggression and an interaction between CU traits and aggression on ROI-based FD were not significant for any of the 12 ROIs examined.

Based on feedback from peer reviewers, we conducted post hoc analyses to determine whether our results may have been influenced by ADHD symptom severity or medication status, and we also tested whether there were any main effects of CU trait score (not covarying for aggression) on FD. Results were unaffected by ADHD severity and medication status, and there were no whole-brain or ROI-specific main effects of

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**Figure 1.** The 12 selected regions of interest (ROIs): left and right uncinate fasciculus (top left); genu (blue), body (green), isthmus (yellow), and splenium (red) of corpus callosum (top right); left and right inferior longitudinal fasciculus (bottom left); left and right cingulum bundle (bottom middle); and left and right fornix (bottom right).

**Figure 2.** Whole-brain analysis revealed group differences in fiber density in children with aggressive behavior vs. control children. Children with aggressive behavior show lower mean fiber density in a cluster of white matter regions when compared with healthy control children. (A) Mean per-subject fiber density values in the significant group-derived cluster are plotted by group. (B) Whole brain significance map demonstrating the group difference in a cluster of white matter fibers within the right hemisphere ventromedial regions. Images are displayed in sample-specific template space. Analyses accounted for the effects of sex, age, IQ, and head motion. Results shown are familywise error-corrected $p < .01$. (C) Sagittal and rotated three-dimensional views of the white matter pathways that overlap with the significant region identified in whole-brain analyses. Post hoc probabilistic tractography analyses showed that the significant cluster overlaps with five distinct white matter tracts in the right hemisphere: the inferior fronto-occipital fasciculus (purple), fornix (blue), middle cerebellar peduncle (red), and superior thalamic radiations (orange and yellow).
parent-rated CU traits. Full details are reported in the Supplement.

DISCUSSION
This study examined associations between aggression and white matter density using a novel diffusion imaging analysis technique (i.e., pixel-based analysis) that leverages connectivity and crossing-fiber information to assess fiber bundle density. Children with aggressive behavior, relative to control children, had lower FD in the bilateral cingulum bundles and a cluster of limbic and cortical pathways, including the inferior fronto-occipital fasciculus, fornix, middle cerebellar peduncle, and superior thalamic radiations. Moreover, among children with aggressive behavior, higher aggression scores were related to higher FD in the corpus callosum. CU traits and anxiety were not uniquely associated with FD at either the whole-brain or ROI level, suggesting that our findings may be specific to aggressive behavior. The results were not affected by covariates, including age, sex, IQ, ADHD symptoms, medications, and head motion.

Consistent with the literature (18,19,21,22,38), we found that children with aggressive behavior had lower FD in the cingulum bundle than control children. The cingulum bundle is a prominent white matter tract positioned directly above the corpus callosum and connects frontal, parietal, and medial temporal regions; it also links subcortical regions to the cingulate gyrus (53). Evidence has suggested that cingulum alterations are associated with impaired attention and cognitive control (53), which are important for emotion regulation.

Moreover, partially consistent with our hypothesis, children with aggressive behavior, relative to control children, had lower FD in key fibers across the association (inferior fronto-occipital fasciculus), commissural (fornix), and projection (middle cerebellar peduncle and superior thalamic radiations) fibers. Decreased white matter density in the inferior fronto-occipital fasciculus (18,22,25) and fornix (17,19,22) has been reported in children with disruptive behavior disorders. The inferior fronto-occipital fasciculus is a ventral associative bundle that connects the ventral occipital lobe and the orbitofrontal cortex (54); it links key regions in the cingulo-opercular network (i.e., the salience network) and the fronto-parietal network (i.e., the executive control network). These networks are critical for the integration of salient emotional and cognitive information needed to allocate attentional resources and promote goal-directed behavior (55,56). The fornix is the main fiber bundle connecting the hippocampus to the hypothalamus, and it

Figure 3. Whole-brain regression analysis in the group of children with aggressive behavior (n = 70) revealed a positive association between aggression and fiber density in the body and splenium of the corpus callosum. Left panel shows the total aggression scores measured by the Child Behavior Checklist Aggressive Behavior Scale plotted against mean per-subject fiber density values in the significant cluster. Sagittal (middle panel) and rotated (right panel) 3-dimensional views of the significant cluster in the body and splenium of the corpus callosum. Results are familywise error–corrected $p < .05$ and accounted for the effects of sex, age, IQ, and head motion.

Figure 4. In a region of interest–based analysis, children with aggressive behavior had significantly lower fiber density in the left cingulum bundle after correcting for multiple comparisons ($\beta = -0.70$, false discovery rate–corrected $p = .03$); this trend was also evident in the right cingulum bundle ($\beta = -0.63$, false discovery rate–corrected $p = .05$). (A) Per-subject fiber density values plotted by subject group. (B) Sagittal, axial, and coronal views of the left cingulum bundle.
arches through the anterior nuclei of the thalamus; it is part of the limbic system and plays a central role in memory formation and retrieval (57). The location of the significant clusters within the corticobulbar tract also suggests that the affected areas are located within the fibers that extend between the substantia nigra in the midbrain through the internal capsule to enervate the caudate and putamen, key regions implicated in reward processing.

White matter alterations in the projection fibers, in contrast to the commissural and association fibers, are less consistently reported in the literature on childhood aggression and disruptive behaviors. This is the first study that found white matter abnormalities (i.e., reduced FA) in the middle cerebellar peduncle and superior thalamic radiations. The middle cerebellar peduncles are the main afferent pathway that connects the cerebellum to the pons (47). It is through this pathway that the cerebellum receives information from the contralateral cerebral hemisphere via the cortico-ponto-cerebellar tract (54), which is implicated in motor tasks, planning, and initiation of movements (58). The superior thalamic radiations are fiber pathways that connect the ventral nuclear group of the thalamus to the primary motor and somatosensory cortices through the superior thalamic peduncle, the posterior limb of the internal capsule, and other parts of the cerebral white matter (59).

Together, these fiber pathways (i.e., the inferior fronto-occipital fasciculus, fornix, middle cerebellar peduncle, and superior thalamic radiations) support the function of many overlapping lower- and higher-order networks that are critical for emotional and reward saliency, executive control, memory, motor functioning, and integration of bodily sensations. Our results suggest that altered or diminished white matter density in these pathways may contribute to risk of aggression in children.

Contrary to our prediction, dimensional analyses at both the whole-brain and ROI levels revealed that among children with aggressive behavior, higher aggression scores were associated with higher FD in the corpus callosum. The corpus callosum is the major white matter commissure of the brain. Intact interhemispheric connectivity through the body of the corpus callosum connecting motor and sensorimotor regions may be critical for enabling higher-order function such as emotion regulation (60). The midposterior part of the corpus callosum (i.e., part of the body and isthmus and splenium) connecting temporoparietal and occipital regions plays an integral role in response inhibition and social processing (61). Increased FA or axial diffusivity in the corpus callosum has been reported in children with disruptive behavior (19,23,26,39), although some other studies have reported decreased FA (22,24). Increased FA is often interpreted as abnormal early or accelerated maturation of neural pathways (15,19,26,62), which could potentially be the case for those in our group of children with aggressive behavior. Early maturation in white matter may reflect processes such as early axonal pruning and early myelination and has been linked to increased risky and dangerous behavior in adolescents (63). It has been hypothesized that early white matter maturation in children with disruptive behavior may be followed by an onset of earlier degeneration (19) as adolescents mature into adults. Future studies with longitudinal designs are warranted to test this hypothesis by mapping out the developmental trajectory of white matter structures linked to aggression. Alternatively, the increased FA in the corpus callosum related to aggression may reflect a potential compensatory mechanism in adaptation to environmental demands. Children with disruptive behavior tend to experience adverse environmental circumstances (e.g., poverty, family stress) earlier and to a greater degree than their peers without disruptive behavior (63). Such adverse experiences may accelerate myelination of the corpus callosum (63). Taken together with other studies, our results suggest that white matter alterations in the corpus callosum may be a neural marker of aggression and disruptive behavior.

Interestingly, we did not find an effect of aggression on white matter microstructure in the uncinate fasciculus, which is a major frontolimbic pathway connecting the amygdala to medial and orbital prefrontal cortex (64), using either a whole-brain or ROI-based approach. This is inconsistent with previous studies in children with disruptive behavior (15,17–20,26,62) and is surprising because of the central role that the uncinate fasciculus plays in the development of antisocial behavior, aggression, and impulsivity (65,66). However, a previous study also failed to identify uncinate abnormalities associated with disruptive behavior (67). Whether our finding reflects a true null finding in the uncinate fasciculus remains to be determined in future research, given the discrepancies between previous positive studies and the current study in terms of sample, phenotypes, age distribution, and DWI analytic approaches.

Of note, in the group of children with aggressive behavior, the associations between aggression and FD in the corpus callosum varied at a trend level of statistical significance as a function of CU traits and anxiety symptoms. It is possible that this lack of moderation effects was due to a relatively low level of CU traits in our sample. Even though our sample included children with high levels of aggression, subjects had no history of serious forms of conduct problems such as use of weapons and criminality. This sample composition was likely affected by recruitment from mental health clinics rather than juvenile justice sources. It is possible that a sample of youths with more serious forms of conduct problems would evidence stronger moderation of aggression neurocircuitry by CU traits. Similarly, the levels of anxiety were low in our sample. It is possible that larger and more heterogeneous samples enriched for high levels of both aggression and anxiety would be more sensitive to detecting the moderating effects of anxiety on the neurocircuitry of aggression.

This study’s strengths include a relatively large sample (compared with most existing studies in children with aggressive behavior), the use of a novel diffusion imaging analysis technique (i.e., fixed-based analysis), and the investigation of the moderating effects of CU traits and anxiety symptoms. Limitations include the cross-sectional design, which precludes us from inferring whether the white matter alterations observed here are a cause or consequence of aggression. In addition, we did not differentiate between forms of maladaptive aggression such as reactive aggression that is unplanned and instrumental aggression that is premeditated and calculated (68,69). These limitations can be addressed in future studies with larger samples.
To conclude, we found that children with aggressive behavior, relative to control children, had lower FD in pathways connecting the limbic and cortical regions, including the inferior fronto-occipital fasciculus, fornix, middle cerebellar peduncle, superior thalamic radiations, and cingulum bundles. In addition, among children with aggressive behavior, higher levels of aggression were associated with higher FD in the corpus callosum. This potentially reflects abnormal interhemispheric connectivity associated with aggression. These results were specific to aggression and not attributed to co-occurring CU traits or anxiety, suggesting a unique neural mechanism of childhood aggression.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the National Institute of Mental Health (Grant Nos. R01MH101514 [to DGS]), RG, KI, and CK are Fellows of the Translational Developmental Neuroscience Training Program (Grant No. T32 MH18268) directed by Michael Crowley. We thank Megan Tudor and Heidi Grantz for subject characterization assessments; Jeffrey Elliott for data management; Spencer McCauley, Emilie Bertschinger, Tess Gladstone, and Carolyn Marsh for study coordination; and Sonia Rowley and Rebecca Jordan for proofreading the manuscript. DGS receives royalties from Guilford Press for a treatment manual on cognitive behavior therapy for anger and aggression in children. All other authors report no biomedical financial interests or potential conflicts of interest.

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