White matter microstructure of the extended limbic system in male and female youth with conduct disorder

Karen González-Madruga[1], Jack Rogers[2], Nicola Toschi[3], Roberta Riccelli[1], Areti Smaragdi[4], Ignazio Puzzo[5], Roberta Clanton[2], Jesper Andersson[6], Sarah Baumann[7], Gregor Kohls[7], Nora Raschle[8], Lynn Fehlbaum[8], Willeke Menks[8], Christina Stadler[8], Kerstin Konrad[7], Christine M. Freitag[9], Stephane A. De Brito[2], Edmund Sonuga-Barke[10], & Graeme Fairchild[11]

[1] Department of Psychology, University of Southampton, Southampton, UK [2] School of Psychology and Birmingham University Imaging Centre, University of Birmingham, Birmingham, UK [3] Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy [4] Centre for Addiction and Mental Health, Toronto, Canada [5]West London Mental Health Trust, Broadmoor High Secure Hospital, London, UK [6]FMRIB, John Radcliffe Hospital, University of Oxford, Oxford, UK [7] Child Neuropsychology Section, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy,University Hospital RWTH Aachen, Aachen, Germany [8] Department of Child and Adolescent Psychiatry,Psychiatric University Clinics and University of Basel, Basel, Switzerland [9] Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany [10] Child and Adolescent Psychiatry Department, Institute of Psychiatry, Psychology and Neuroscience, Kings College London, London, UK [11]Department of Psychology, University of Bath, Bath, UK

*Address for correspondence:
Karen Gonzalez-Madruga
Department of Child and Adolescent Psychiatry,
Institute of Psychiatry, Psychology and Neuroscience,
Kings College London,
London, UK
Email: Karen.gonzalez.madruga@gmail.com

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Abstract

Background: Previous studies of conduct disorder (CD) have reported structural and functional alterations in the limbic system. However, the white matter tracts that connect limbic regions have not been comprehensively studied. The uncinate fasciculus (UF), a tract connecting limbic to prefrontal regions, has been implicated in CD. However, CD-related alterations in other limbic tracts, such as the cingulum and the fornix, have not been investigated. Furthermore, few studies have examined the influence of sex and none have been adequately powered to test whether the relationship between CD and structural connectivity differs by sex. We examined whether adolescent males and females with CD exhibit differences in structural connectivity compared to typically-developing controls.

Methods: We acquired diffusion-weighted MRI data from 101 adolescents with CD (52 females) and 99 controls (50 females). Data were processed for deterministic spherical deconvolution tractography. Virtual dissections of the UF, the three subdivisions of the cingulum (retrosplenial, parahippocampal and subgenual cingulum), and the fornix were performed and measures of fractional anisotropy (FA) and hindrance-modulated orientational anisotropy (HMOA) were analysed.

Results: The CD group had lower FA and HMOA in the right retrosplenial cingulum tract relative to controls. Importantly, these effects were moderated by sex - males with CD significantly lower FA compared to male controls, whereas CD and control females did not differ.

Conclusions: Our results highlight the importance of considering sex when studying the neurobiological basis of CD. Sex differences in retrosplenial cingulum connectivity may contribute to sex differences in the clinical presentation of CD.

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Introduction

Conduct Disorder (CD) is diagnosed in children and adolescents who display a pattern of behaviour in which societal rules and the rights of others are violated (American Psychiatric Association, 2013). Although the lifetime prevalence of CD is higher amongst males than females (by a ratio of approximately 2.4:1), it is increasingly prevalent in adolescent females. Individuals with CD have poor prognoses with negative adult outcomes that include criminality, alcohol abuse, unemployment, and poor mental and physical health. CD is one of the main reasons for referral to child and adolescent mental health services, and places a high burden on the affected individuals, families and society in general. Therefore, CD can be considered a major mental and public health priority and gaining a better understanding of its neurodevelopmental underpinnings is critical.

It has been proposed that limbic system dysfunction may underlie antisocial behaviour. Brain regions that make up the limbic system include the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC), orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC), hippocampus, hypothalamus, amygdala, and medial temporal lobe (Rolls, 2004, 2013). The limbic system is involved in emotion processing and regulation, reward-related decision-making and a range of other cognitive functions (Blair, 2008). Evidence implicating limbic brain structures in antisocial behaviour comes from a number of sources. Two structural magnetic resonance imaging (sMRI) meta-analyses concluded that the most robust abnormalities in grey matter volumes observed in this population are in limbic brain structures, such as the amygdala, ACC and vmPFC (Aoki et al., 2014; Raschle et al., 2015; Rogers and De Brito, 2016). In line with this, a recent meta-analysis of fMRI studies reported that CD individuals consistently displayed underactivation in the ACC and vmPFC during tasks involving emotion processing, and 'hot' (motivationally-relevant) executive functions, and in dorsolateral prefrontal cortex (dlPFC), dorsal ACC, and hippocampus during 'cool' (non-affective) executive function tasks (Alegria et al., 2016).

Given this evidence for structural alterations and abnormal neural activity in limbic regions in individuals with CD, it is possible that the structural connections linking these regions are also compromised. The major limbic system white matter (WM) pathways include the fornix, the cingulum, and the uncinate fasciculus (UF; Catani et al., 2013). Structural connectivity and the micro-structural properties of brain tissue are frequently assessed using diffusion tensor imaging (DTI) techniques (Catani and Thiebaut de Schotten, 2012). However, previous DTI studies in youths with CD and related disorders have had several limitations. First, the majority of DTI studies in CD have focused on males (although see Menks et al., 2017). Therefore, possible sex differences in the microstructural integrity of limbic system-related tracts have not been investigated. This is important as the neurobiological basis of CD has been shown to differ in several respects between males and females (Fairchild et al., 2013;

Decety et al., 2015; Smaragdi et al., 2017). Only one small study directly compared males and females with CD (n=14 and 13, respectively) in terms of WM microstructure (Zhang et al., 2014). It investigated fractional anisotropy (FA) values of the UF using deterministic tractography (which investigates specific anatomical pathways). Interestingly, the authors found higher FA values in the UF in males, but not females, with CD. These preliminary findings suggest that WM microstructural alterations in temporo-frontal regions might be specific to males with CD.

Additionally, aside from one very recent study (Sethi et al., 2018), previous studies using DTI-based tractography methods in individuals with CD have largely focused on the UF tract (Passamonti et al., 2012; Sarkar et al., 2013; Zhang, Gao, et al., 2014). The fact that these studies focused on this tract may have been due to earlier studies in adults with antisocial personality disorder (ASPD; an adult condition of which CD is an antecedent) and psychopathy, finding lower FA in the UF in these individuals compared to healthy controls (Craig et al., 2009). However, opposite findings have been reported in youths with CD, who show higher FA values in the UF relative to healthy controls (Passamonti et al., 2012; Sarkar et al., 2013; Zhang, Gao, et al., 2014). Similarly, a study investigating the dorsal and ventral components of the cingulum tract, reported lower radial diffusivity (RD) in the dorsal cingulum bundle in individuals with CD compared to controls (Sethi et al., 2018). This was opposite to the pattern observed in adults with ASPD (Sethi et al., 2014). Several authors have suggested that the opposite patterns observed in WM microstructural measures in youths and adults might be due to abnormally accelerated maturation of WM tracts in individuals with CD (Passamonti et al., 2012; Sarkar et al., 2013b; Zhang, Gao, et al., 2014). Although these previous studies were important first steps in understanding CD-related alterations in structural connectivity, the focus of research needs to be expanded to consider additional limbic system tracts. It is also important to test whether alterations in WM microstructure are common or distinct across males and females with CD.

An additional limitation of prior studies is that they used either tractography methods or a characterization of WM diffusivity exclusively based on the diffusion tensor model. Although the diffusion tensor model is most frequently used to reconstruct WM tracts and characterise diffusivity in WM (Basser et al., 2000), this approach has several limitations. First, it is not well-suited for studying complex fibre configurations such as crossing fibres, branching regions or intra-voxel combinations of different tissue types (e.g., WM fibres and grey matter). Second, while FA is the most commonly used index to quantify water diffusivity in studies using tensor-based models, it is calculated at a voxel level and is determined by several microstructural and macrostructural features, such as myelination of WM fibres, size and packing density of cells and number of crossing fibres (Vanderauwera et al., 2015). Thus, partial volume effects (i.e., not fibre- or tissue -specific) can affect DTI indices (e.g., FA, RD), and voxel-average diffusion MRI parameters such as FA, lack within-voxel single fibre population specificity (Dell'Acqua et al., 2013; Raffelt et al., 2015). Novel non-tensor models such as constrained

spherical deconvolution (SD) have the potential to overcome these limitations and more accurately characterise the underlying architecture of specific WM tracts (Dell'Acqua et al., 2010). In addition, the hindrance-modulated orientational anisotropy (HMOA) index that can be derived using SD algorithms provides greater sensitivity in terms of detecting microstructural changes in specific WM tracts than FA (Dell'Acqua et al., 2013). Finally, most previous DTI studies included relatively small samples - typically groups of 15 participants or fewer (Sethi et al., 2018; Finger et al., 2012; Haney-Caron et al., 2014; Passamonti et al., 2012; Zhang, Gao, et al., 2014).

The present study addresses a number of these limitations, and extends previous findings by, first, examining sex differences in the relationship between CD and WM microstructure. Second, by examining two key limbic WM tracts overlooked in prior studies: the fornix and the cingulum bundles - the retrosplenial (RSC), parahippocampal (PHC) and subgenual cingulum (SGC; Jones et al., 2013) - as tracts plausibly involved in the pathophysiology of CD. Third, by enhancing statistical power and the robustness of our results by substantially increasing the sample size compared to previous studies. Finally, by employing a novel method – constrained SD. Recent studies have compared tensor versus non-tensor models in clinical samples and suggested that the latter approach provides more accurate and robust results (Auriat et al., 2015). However, to increase comparability with previous studies, we also estimated indices of FA - the most widely-used parameter in previous structural connectivity research.

We hypothesised that differences between CD and control groups would be most evident in limbic tracts involved in socio-emotional processes (i.e., subgenual cingulum, retrosplenial cingulum, and UF) in comparison with posterior and lateral limbic WM tracts (e.g., parahippocampal cingulum). We also hypothesised that CD-related alterations in WM microstructure would be most evident in males (Zhang et al., 2014). In addition, recent DTI studies have shown that individuals with CD and elevated callous-unemotional (CU) traits may differ from those with low levels of CU traits in terms of WM microstructural abnormalities (Sethi et al., 2018; Puzzo et al., 2017). Thus, we also investigated whether CU traits contributed to the WM microstructural alterations observed in CD. We also tested for correlations between WM measures and the grandiose-manipulative and impulsive-irresponsible subdimensions of psychopathy and CD symptoms.

Methods

Participants

Participants for this study were recruited at four different sites involved in the Neurobiology and Treatment of Female Conduct Disorder (FemNAT-CD; www.femnat-cd.eu) study - University of

Southampton, University of Birmingham, University Hospital Aachen, and University of Basel. All participants and the majority of their parents underwent a diagnostic interview that was based on DSM-IV criteria (the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime; Kaufman et al., 1997). At the UK sites, IQ was assessed using the two subtest form of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999) whereas the German version of the Wechsler Intelligence Scale for Children (Wechsler, 2003) was used at the other sites. The t and standard scores from all sites were transformed into z-scores and then combined to yield estimates of full-scale IQ.

The Match program (Van Casteren and Davis, 2007) was used to select an IQ-, age- and gender-matched sample from the subset of participants for whom diffusion MRI data were available (n=325). There are dramatic changes in WM development across childhood and adolescence (Casey et al., 2008), thus we excluded children aged 9-12 years (n=57; see Supplementary Material for more information). A total sample of 200 adolescents (101 with conduct disorder (52 females) and 99 healthy controls (50 females) was included in the present analyses – all aged 13-18 years. The Youth Psychopathic traits Inventory (YPI; Andershed et al., 2002); a self-report questionnaire assessing overall psychopathic traits and sub-dimensions of psychopathy, and the parent-report Inventory of Callous Unemotional traits (ICU; Essau, Sasagawa and Frick, 2006); a standardized measure including callous, uncaring and unemotional subscales, were used to assess psychopathic and callous-unemotional (CU) traits, respectively.

Diffusion-weighted MRI data acquisition

Diffusion-weighted MRI data were acquired with the following parameters: Repetition time (TR) = 8000ms (Achieva), 8800ms (Tim-Trio), 7500ms (Prisma); echo-time (TE) = 87ms (Achieva), 92ms (Tim-Trio), 71ms (Prisma) and a bandwidth of 1633.3 Hz/Px (Achieva) or 1776 Hz/Px (Tim-Trio & Prisma); echo-spacing = 0.75ms (Achieva), 0.73ms (Tim-Trio), 0.65ms (Prisma); slice thickness = 2.0mm; field of view (FOV) = 256 x 256 x 124mm; acquisition matrix = 128 x 128; voxel-size = 2×2×2mm; 62 contiguous axial slices (no slice gap). Images were acquired with diffusion gradients (b-value=1500 s/mm²) applied in 64 non coplanar and non collinear directions and two b-value=0 (s/mm²) volumes with reversed phase encoding (blip-up/blip-down), yielding pairs of images with distortions in opposite phase-encode directions to enable accurate estimation and correction for susceptibility-induced distortions.

Pre-processing

Datasets were corrected for head motion and eddy current distortions using FSL (Andersson and Sotiropoulos, 2016). Distortions in the magnetic field were estimated. The estimated field was subsequently used, together with all the diffusion data, to estimate eddy current-induced distortions and subject movement (Andersson and Sotiropoulos, 2016). Movement-induced signal dropout was identified and the lost signal was replaced by a non-parametric Q-space interpolation (Andersson et al., 2016).

Spherical deconvolution was calculated using the damped Richardson-Lucy algorithm (Dell'Acqua et al., 2010) with a fibre response parameter of α =1.5, 400 algorithm iterations, threshold parameters of 0.06, and a harmonic order of 8 (lmax=8). An absolute (0.1%) and relative (5%) threshold on the Fibre Orientation Distribution (FOD) amplitude were applied to exclude spurious local connections (Dell'Acqua et al., 2013).

Whole brain deterministic tractography was performed using a step size of 0.5mm with a limit set to display streamlines between 20 and 400 mm. The Euler algorithm was used to follow the orientation vector of least curvature (angle threshold of 45º). Spherical deconvolution and tractography analysis was performed using StarTrack software (https://www.mr-startrack.com/). Explore DTI was used for the tensor fit. Tensor-derived FA and HMOA values of WM microstructural organisation were exported to TrackVis. We report FA values in the present study for the purpose of increasing comparability with previous studies.

Delineation of Regions of Interest

TrackVis was used to reconstruct the fornix, cingulum bundle subdivisions and UF. Reconstruction of these tracts has previously been described (fornix and UF: (Stieltjes et al., 2013), and CB subdivisions: (Jones et al., 2013)). The Boolean logic (AND, and NOT gates) was employed to delineate the CB´s subdivisions, the fornix and the UF (Figure 1 shows the reconstruction for all of the limbic WM tracts).

Statistical analysis

Matlab_R2016 was used to carry out statistical analysis. Shapiro-Wilk tests were used to verify normality of HMOA and FA values across subjects.. Tract measures of HMOA and FA were analysed using a general linear model (GLM) to test for effects of diagnosis and sex-by-diagnosis interactions. The GLM included the following covariates which have been shown to be associated with WM microstructural integrity in adolescent studies: age (Asato et al., 2010), IQ (Dunst et al., 2014), and site (coded as binary fixed effect). Where significant sex-by-diagnosis interactions were found, we followed these up by comparing FA and HMOA values between CD and healthy control males, and between CD and healthy control females.

ADHD is a neurodevelopmental disorder that frequently co-occurs with CD, and previous DTI studies have shown that comorbid ADHD strongly modulates WM effects (Wang et al., 2012). Thus, we repeated the GLM analysis while adding current ADHD symptoms (i.e., those displayed in the last year) as an additional covariate.

In addition, there is some evidence for structural differences between the childhood–onset (CO) and adolescent-onset (AO) variants of CD (Fairchild et al., 2015). Accordingly, we used the same model to compare these subgroups, to assess the validity of combining these subgroups in our main analysis.

The significance threshold was adjusted using the Benjamin–Hochberg false discovery rate (FDR: $q<0.05$) correction for multiple comparisons across each parameter independently. Effect sizes for diagnosis effects were calculated using Cohen's d and effect sizes for sex-by-diagnosis interactions were expressed as partial eta-squared (ηp^2).

In cases where significant main effects of diagnosis were observed, we followed these up by running a GLM analysis (only in the CD group), to test for associations between CD symptoms, psychopathy (YPI total), dimensions of psychopathic traits (grandiose-manipulative, and impulsive-irresponsible traits), CU traits (YPI subscale and ICU total), ICU subscales (callousness, uncaring, unemotional), and measures of HMOA and FA. Lastly, given that the CD sample included many individuals with comorbid ADHD, we also explored the relationship between ADHD symptoms and measures of HMOA and FA. These correlational analyses were run in a mixed-sex CD group (males and females with CD) as well as in each sex separately.

Results

Demographic Variables

Individuals in the CD group had significantly more CD, ODD, and ADHD symptoms than healthy controls. They also scored higher in overall psychopathic traits, as well as callous-unemotional, grandiose and manipulative, and impulsive and irresponsible subdimensions of psychopathy (Table 1). There were no significant differences between males and females in the age-of-onset of CD (i.e., childhood-onset vs. adolescent-onset).

In terms of psychiatric comorbidity in individuals with CD, males and females differed only in rates of substance abuse (M>F); there were no other significant differences between males and females. Finally,

there was an unequal sample distribution across the sites (see Supplementary Table 1). To address this issue, we included site as a covariate of no interest.

Tractography results

There were no significant differences between the CO-CD and AO-CD subtypes in HMOA or FA in any WM tract.

Main effects of diagnosis

Relative to controls, individuals with CD had lower HMOA in bilateral retrosplenial cingulum (RSC; right: t(190)=-2.22, p=0.03, d=0.10; left: t(190)=-2.27, p=0.02, d=0.16), and lower FA in the right RSC (t(190)=-2.91, p=0.004, d=0.28). However, after correcting for multiple comparisons, only the effect on right RSC FA remained significant (pFDR=.03; Figure 2). There were no significant group differences in HMOA or FA in any of the other limbic WM tracts.

Sex-by-diagnosis interactions

We observed sex-by-diagnosis interactions for HMOA in bilateral RSC (right: t(190)=2.08, p=0.04, ηp^2=0.02: left: t(190)=1.99, p=0.05, ηp^2=0.02), and FA in right RSC (t(190)=2.75, p=0.006, ηp^2=0.04). All interactions followed the same pattern: males with CD showed lower values than male controls, whereas females with CD showed higher values than female controls (Figure 3). However, only the sex-by-diagnosis interaction for right RSC FA survived correction for multiple comparisons (pFDR=.05). No other significant interaction effects were found in the other limbic tracts (see Supplementary Table 2). Post-hoc analysis showed that relative to male controls, CD males had lower HMOA in bilateral retrosplenial cingulum (RSC; right: t(190)=-2.52, p=0.04, d=0.39; left: t(190)=-1.99, p=0.04, d=0.37) and lower FA in the right RSC (t(190)=-2.91, p=0.01, d=0.47). However, after correcting for multiple comparisons, only the effect in right RSC FA remained significant (pFDR=.03). There were no significant differences between female CD and control groups.

ADHD comorbidity as a potential confound

The main effects of diagnosis observed for FA in the right RSC (p=0.03) and for HMOA in bilateral RSC (left: p=0.05: right: p=0.05) in CD versus healthy control males remained significant after factoring out current ADHD symptoms. However, only the group difference in right RSC FA remained significant (pFDR=0.03) after correcting for multiple comparisons. Moreover, significant main effects of diagnosis emerged in the right UF when factoring out ADHD symptoms: participants with CD showed lower FA (t(189)=2.00, p=0.05, pFDR=0.05), and HMOA (t(189)=2.07, p=0.04, pFDR=0.05;

Figure S1) relative to healthy controls. Unlike the findings for the RSC, this main effect of diagnosis in the UF was not qualified by a significant sex-by-diagnosis interaction.

Correlations between structural connectivity measures and CD symptoms, ADHD symptoms, and psychopathic or callous-unemotional traits

Within the CD sample, there was a positive correlation between current CD symptoms and right RSC HMOA (r=.36, pFDR=0.02; Figure 4). There were no other significant correlations between CD, ADHD symptoms, overall psychopathic traits, the subdimensions of psychopathy, CU traits or the ICU subscales (Callousness, Uncaring, Unemotional) and measures of WM in other tracts.

As effects of diagnosis were found in males, but not in females with CD, we conducted correlational analyses in males and female groups separately. A strong positive correlation between current CD symptoms (r=.45, pFDR=0.002; Figure S2), and a negative correlation between current ADHD symptoms (r= -.31, pFDR=0.03; Figure S3) and right RSC HMOA was found in the male CD group. No effects of CU or psychopathic traits were observed in CD males. No significant correlations were found between clinical symptoms or CU/psychopathic traits or subscales and measures of structural connectivity in females with CD. Moreover, there were no significant sex-by-CD symptoms or sex-by-CU/psychopathic traits interactions for either HMOA or FA.

Discussion

Abnormalities in the limbic system have been consistently implicated in the pathophysiology of CD (Alegria et al., 2016; Raschle et al., 2015; Rogers and De Brito, 2016). We extended the DTI literature by including female participants and a much larger sample than has been studied to date (N=200). This allowed us to test whether females and males with CD show common or distinct alterations in limbic WM microstructure. We also investigated limbic WM tracts beyond the UF and capitalised on recent methodological advances in diffusion-weighted image processing by employing spherical deconvolution (SD) models. This approach provides a more reliable estimation of multiple fibres passing through a voxel with distinct orientations.

Our findings extend knowledge regarding alterations in limbic WM tracts in CD and support the hypothesis that abnormalities in fronto-limbic tracts are involved in the pathophysiology of this disorder. However, such abnormalities appear to be limited to males with CD – no such effects were found in females. More specifically, only males with CD showed lower FA in the right RSC relative to male controls. In fact, there was a suggestion that the opposite pattern was observed in females (females

with CD appeared to show higher FA and HMOA values relative to control females) – although this was not statistically significant.

Previous DTI studies have found structural abnormalities in regions that overlap with the RSC. A recent study using a similar approach to the present study (i.e., region of interest-based tractography) investigated dorsal and ventral cingulum WM microstructure in male youths with CD (Sethi et al., 2018). Lower RD values were observed in bilateral dorsal cingulum in the CD group relative to controls (Sethi et al., 2018). FA values normally increase when RD decreases, and the opposite pattern seems associated with myelin loss and axonal abnormalities (Harsan et al., 2006). Although the anatomical delineation of the cingulum bundle differed between the two studies (i.e., dorsal and ventral in Sethi et al. versus retrosplenial, parahippocampal and subgenual cingulum in the present study), the dorsal part of the cingulum overlaps most closely with the RSC tract compared to the other cingulum bundles – thus the findings are congruent in terms of location, but not in the direction of the effects. In addition, the Sethi et al. (2018) study differs from the present study in the use of tensor-based models versus non-tensor models.

The RSC is composed of fibres that connect the medial prefrontal cortex, dlPFC, ACC, PCC, medial temporal lobe, and angular gyrus together (Jones et al., 2013). These regions have been associated with social-emotional processing, self-reflection, executive functions and moral decision-making. They are key nodes of the default mode network (DMN) that is responsible for self-referential processing (Leech et al., 2012). Previous studies investigating DMN connectivity in youths with CD have reported reduced connectivity between core DMN regions including the medial PFC, PCC, precuneus and superior temporal gyrus, relative to controls (Broulidakis et al., 2016; Zhou et al., 2016). It has been proposed that DMN dysfunction in CD may reflect delays in the development of brain circuits linked to self-awareness, regulating emotions, moral judgments and future planning (Zhou et al., 2015). Impairments in these processes have been reported in CD (e.g. White et al., 2014). The RSC connects core regions that make up the DMN. Thus, the abnormal functional connectivity of the DMN observed in previous studies may have a structural basis in altered RSC connectivity.

Although group differences in FA in the UF only became significant after controlling for comorbid ADHD symptoms, our results are in contrast to findings reported by Zhang et al. (2014). We did not observe any sex-by-diagnosis interactions in this WM tract. Both males and females with CD appeared to be equally affected in terms of showing lower UF FA. However, in line with Zhang et al. (2014), we also observed sex differences in the RSC tract in youths with CD. Males with CD showed lower FA (and HMOA at an uncorrected level) relative to sex-matched healthy controls, whereas there were no significant differences between CD and control females.

Previous DTI studies of CD have observed higher FA values in male-only samples, suggesting accelerated maturation in individuals with CD. Here, we observed lower FA in males with CD compared to male controls. Although the results observed here were in a previously unstudied tract, it suggests that WM maturation is delayed in males with CD. Delayed maturation of WM is associated with poor inhibitory control (Simmonds et al., 2014) - a key feature of CD.

Furthermore, our correlational analyses showed that CD symptoms were significantly (positively) correlated with HMOA of the right RSC tract in males, but not females. Therefore, the present study provides new evidence for sex differences in the neurobiological basis of CD – RSC WM abnormalities were observed in males but not females. We also observed a significant negative correlation between ADHD symptoms and HMOA in the right RSC tract in males but not females, indicating that ADHD comorbidity may have influenced the differences between CD and control males in the RSC. This is of significance due to the substantial overlap of ADHD and CD, and symptom dimensions related to ADHD such as impulsivity and hyperactivity have been associated with the development of antisocial behaviour in childhood (Barkley et al., 2004).

Several neuropsychological studies investigating aspects of executive functioning (i.e., assessing inhibition/attention and decision-making), are consistent with our findings by showing divergent results in males and females with CD. Males with CD exhibit deficits in reversal learning (Herpertz et al., 2008) and differ in terms of decision-making (e.g., making more risky choices) relative to control males, whereas CD females do not differ from control females (Sidlauskaite et al., 2017). In addition, our finding of a sex-by-diagnosis interaction in the RSC highlights the importance of taking sex into account when studying the neurobiology of CD, and the problems that might arise when combining males and females with CD in the same group (Smaragdi et al., 2017). Future studies should investigate the functional consequences of altered RSC structural connectivity in males and females with CD by employing resting state functional connectivity methods, and by using neuropsychological tasks tapping decision-making and empathic processes in the same sample.

Strengths and limitations

The strengths of this study include the investigation of additional limbic WM tracts by using a more comprehensive approach – SD tractography. The main benefit of this approach is to resolve fibre-crossing issues. In addition, SD techniques improve the accuracy of fibre tracking compared to models based on the diffusion tensor alone (Dell'Acqua et al., 2010). Secondly, the comparatively large sample size in the present study (N=200), which included males and females with and without CD, allowed us, for the first time, to comprehensively investigate sex differences in the relationship between CD and structural connectivity. Another strength is the fact that the CD group was assessed using standardised,

semi-structured interviews based on DSM-IV criteria as well as obtaining detailed information about comorbid disorders and accounting for ADHD comorbidity in our statistical analyses.

However, our study also had several limitations. First, the sample ranged in age from 13-18 years. The CD and control groups did not differ in age; however, age is known to have an important effect on white matter development. Thus, we included age as a covariate of no interest in all analyses. Second, the sex distribution across the sites was uneven (more girls were tested at some sites than others), and although quality control procedures were performed prior to starting data acquisition (e.g., matching acquisition parameters and going through a site qualification process), combining data from different sites and scanner manufacturers (Phillips and Siemens) may introduce unintended variability. However, to reduce the impact of this variability, all analyses included site as a covariate of no interest. Finally, although we used SD methods to reconstruct the WM tracts, indices of FA were derived from tensor-based models fitted to b=1500 diffusion-weighted data and projected onto the SD-derived tracts. Hence there is a potential source of variability in terms of comparing the present FA measures with those reported in previous studies, although several earlier studies adopted a similar approach (e.g., Christiansen et al., 2016, Rojkova et al., 2016).

In conclusion, we found that male adolescents with CD differed from healthy controls in retrosplenial cingulum white matter microstructure – showing lower FA and HMOA values in this tract. This effect was not seen in females with CD. These differences in structural connectivity may help explain sex differences in CD and its clinical presentation. Given the overlap of the RSC tract with brain regions that constitute the DMN, and its role in connecting these regions together, future studies should investigate whether there are sex differences in DMN functional connectivity in CD. This would improve our understanding of the pathophysiology of CD and could lead to improved diagnosis and treatments for both sexes.

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Conflicts of interest

Prof. Freitag receives royalties for books on Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder. She has served as consultant to Desitin and Roche. Prof. Sonuga-Barke has received speaker fees, consultancy, research funding and conference support from Shire Pharma. Speaker fees from Janssen Cilag, consultancy from Neurotech solutions, Aarhus University, Copenhagen University and Berhanderling, Skolerne, Copenhagen, KU Leuven. Book royalties from OUP and Jessica Kingsle. Edmund Sonuga-Barke has been awarded grants from the MRC, ESRC, Wellcome Trust, Solent NHS Trust, European Commission, Child Health Research Foundation New Zealand, NIHR, Nuffield Foundation, Fonds Wetenschappelijk Onderzoek-Vlan-deren (FWO), and MQ—Transforming Mental Health. Dr. Fairchild has received funding from the European Commission, the UK Medical Research Council, the National Council for Science and Technology (CONACYT), the UK Economic and Social Research Council and Kids' Company. Prof. Konrad has received speaker fees from Shire Pharmaceuticals and Medice. Prof. Stadler receives royalties for a book on aggression. Dr. De Brito has received speaker fees from the Child Mental Health Centre and the Centre for Integrated Molecular Brain Imaging. All other co-authors declare no potential conflicts of interest.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1 Demographic and clinical characteristics of the sample

Variable	Females (Mean±SD)		Males (Mean±SD)		Statistics		
	CD (n = 52)	HC (n = 50)	CD (n = 49)	HC (n = 49)	Diagnosis F(*p*)	Sex F(*p*)	Sex x Diagnosis F(*p*)
Age (years)	15.44±1.64	15.38±1.66	15.38±1.765	15.34±1.80	0.045 (0.83)	0.032 (0.85)	0.002(0.96)
Estimated IQ	99.48±11.58	100.24±12.20	96.16±9.49	97.43±11.58	0.403 (0.53)	3.69 (0.06)	0.025 (0.87)
CD symptoms (K-SADS-PL)	5.00±2.89	0.06±0.23	5.53±2.73	0.14±0.40	328.50 (0.001)	1.15 (0.28)	0.617 (0.43)
ODD symptoms (K-SADS-PL)	5.61±2.94	0.02±0.14	5.12±2.92	0.08±0.40	321.26 (0.001)	0.53 (0.47)	0.873 (0.35)
ADHD symptoms (K-SADS-PL)	5.37±5.91	0.08±0.44	7.06±6.58	0.02±0.14	96.04 (0.001)	1.69 (0.19)	1.94 (0.16)
#PTSD (No. traumatic events)	2.85±1.98	1.06±1.17	2.59±2.02	1.41±1.19	41.03 (0.001)	0.013 (0.91)	14.22 (0.001)
CD age of onset – No. (%)							
Childhood onset	24(46)		28(58)			X^2=1.22 (0.26)	
Adolescent onset	28(54)		21(43)				
Handedness - No. (%)							
Right	45(87)	40(80)	40(82)	46(94)	X^2= 2.04(0.56)	X^2= 1.54(0.67)	X^2= 9.70(0.37)
Left	2(4)	8(16)	7(15)	2 (4)			
Ambidextrous	3(6)	0	1(2)	0			
Missing	2(4)	2(4)	1(2)	1 (2)			
Psychological and personality measures							
Callous subscale (ICU)	10.56±5.31	5.06±3.06	10.43±5.78	5.82±3.06	61.63(0.001)	2.30(0.63)	0.47(0.50)
Uncare subscale (ICU)	14.52±4.98	6.60±3.32	15.27±4.52	9.29±5.50	125.47 (0.001)	7.52(0.01)	2.45(0.12)
Unemotional subscale (ICU)	7.54±4.09	4.36±2.45	7.43±2.76	6.10±3.06	25.24(0.001)	3.18 (0.08)	4.26(0.05)
Total ICU	33.87±12.61	14.66±5.32	34.10±10.92	19.80±8.40	146.97(0.001)	3.69 (.06)	3.15(0.08)
Grandiose manipulative (YPI)	38.13±9.34	31.92±9.09	38.18±13.07	34.93±9.09	10.59 (0.001)	1.11 (0.29)	1.044 (0.30)
Callous/Unemotional (YPI)	29.73±7.69	26.44±6.03	35.57±10.43	30.36±5.29	15.53 (0.001)	20.54 (0.001)	0.788 (0.37)
Impulsive/Irresponsible (YPI)	41.19±8.12	32.16±6.74	39.55±10.01	32.85±5.90	50.12 (0.001)	0.18 (0.67)	1.108 (0.29)
Total YPI	109.07±20.86	90.52±17.89	113.30±28.43	98.18±15.93	31.24 (0.001)	3.89 (0.05)	0.324 (0.57)
Current psychiatric comorbidity - No. with K-SADS-PL diagnoses (%)							
ADHD	16(31)	-	21(43)	-		X^2=1.58(0.20)	
ODD	34(65)	-	30(61)			X^2=0.18 (0.66)	
PTSD	7(14)		2(4)			X^2= 2.73 (0.09)	
MDD	11(21)		4(8)			X^2= 3.36 (0.06)	
Alcohol abuse	1(2)		4(8)			X^2=2.08 (0.14)	
Alcohol dependence	0		0				
Substance abuse	1(2)		7(14)			X^2=5.28 (0.02)	
Substance dependence	1(2)		5(10)			X^2=3.09 (0.07)	
Generalized Anxiety Disorder	7(14)		2(4)			X^2= 2.73 (0.09)	

Note: CD, Conduct disorder; HC, healthy controls; ADHD, attention-deficit/hyperactivity disorder; ODD, Oppositional defiant disorder; PTSD, post-traumatic stress disorder; MDD, major depressive disorder; YPI, Youth Psychopathic traits Inventory; ICU, Inventory of Callous Unemotional traits. # Number of traumatic events were estimated from the K-SADS-PL interview data.

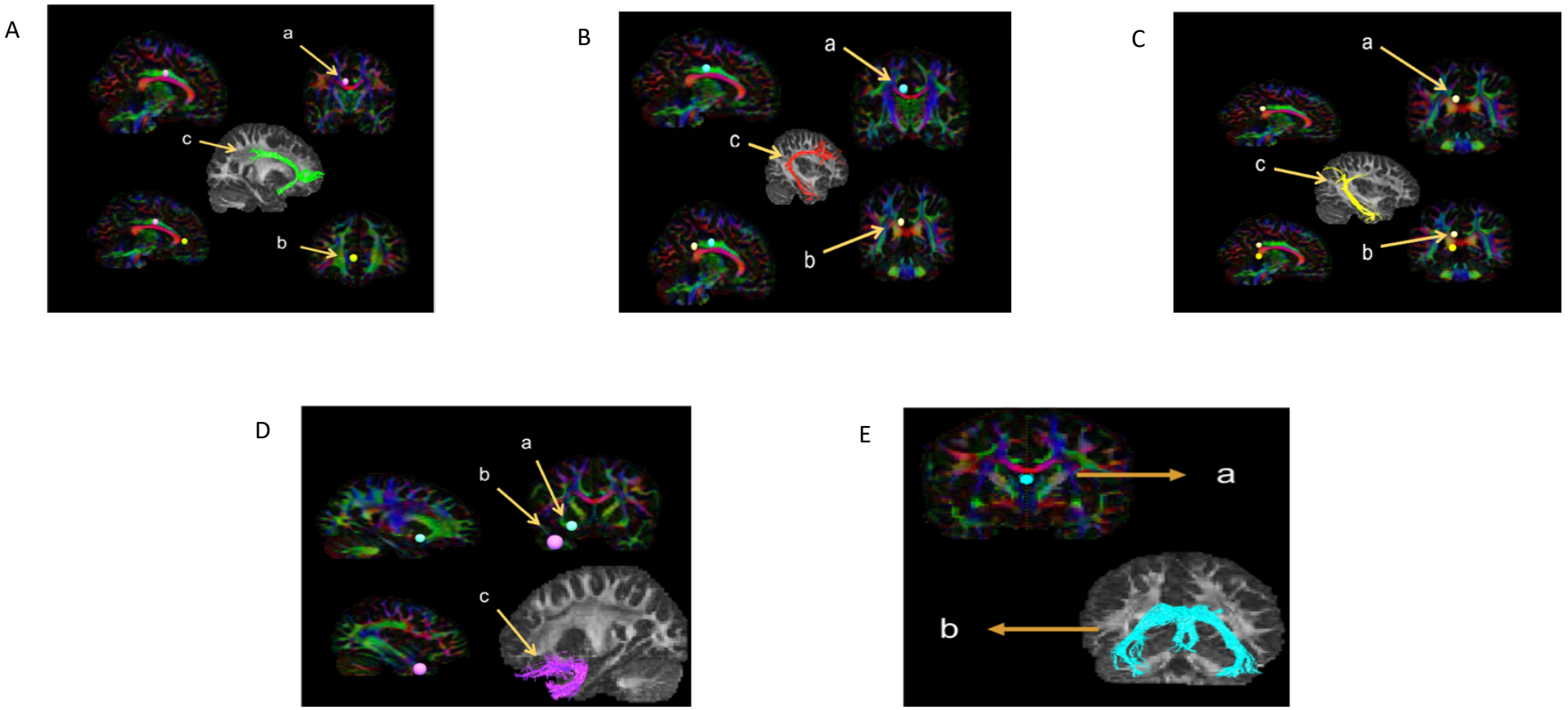

Figure 1 Tractography reconstruction for all limbic white matter tracts. **A**. **Coronal tractography reconstruction of the subgenual cingulum (SGC):** a) First sphere of interest placed 5 slices anterior to the mid-sagittal view. b) Target sphere placed 3 slices anterior to the genu of the corpus callosum. c) Final reconstruction of the SGC. **B. Coronal tractography reconstruction of the retrosplenial cingulum (RSC):** a) First sphere of interest placed 5 slices posterior to the mid-sagittal view. b) Target sphere placed 3-4 slices above the splenium. c) Final reconstruction of the RSC. **C. Coronal tractography reconstruction of the parahippocampal cingulum (PHC).** a) First sphere of interest placed behind the splenium. b) Target sphere placed 3-4 slices below sphere A. c) Final reconstruction of the PHC. **D. Coronal tractography reconstruction of the uncinate fasciculus (UF)**: a) First sphere of interest placed in the fronto-temporal junction. b) Target sphere placed at the temporal pole. c) Final reconstruction of the UF. **E. Coronal tractography reconstruction of the fornix.** a) One coronal sphere placed in the body of the fornix. b) Final reconstruction of the fornix.

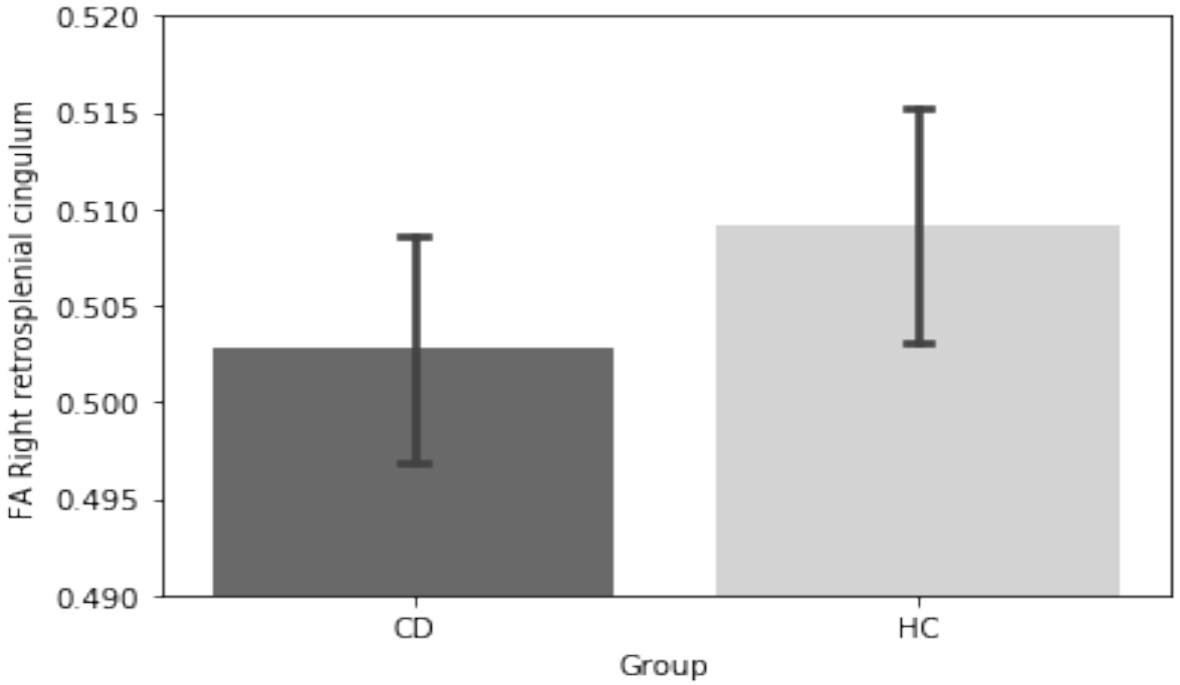

Figure 2. Main effects of diagnosis on fractional anisotropy of the right retrosplenial cingulum. Group differences are significant at p<.05, False Discovery Rate correction. Error bars show 95% confidence intervals of the mean. HC, healthy control; CD, Conduct Disorder; FA, fractional anisotropy

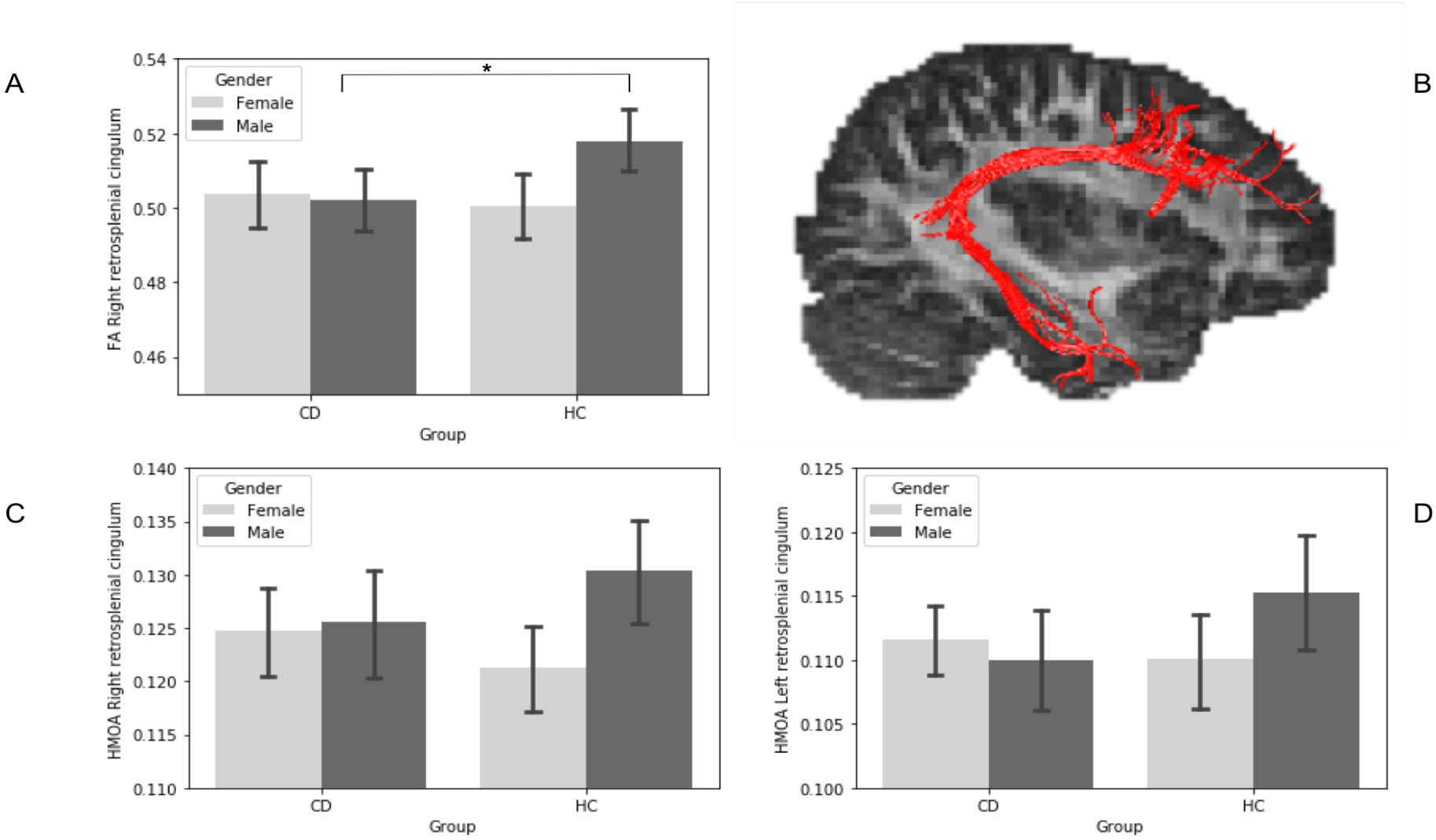

***Figure* 3. Sex by diagnosis interactions in the retrosplenial cingulum tract**. A) Sex by diagnosis interaction on fractional anisotropy values in the right retrosplenial cingulum, which was significant at pFDR=0.05. B) 3D reconstruction of the retrosplenial cingulum tract. C) Sex by diagnosis interactions in the Hindrance Modulated Orientational Anisotropy of the right retrosplenial cingulum. D) Sex by diagnosis interactions in the Hindrance Modulated Orientational Anisotropy of the left retrosplenial cingulum. Error bars show 95% confidence intervals of the mean. HC, healthy control; CD, Conduct Disorder.

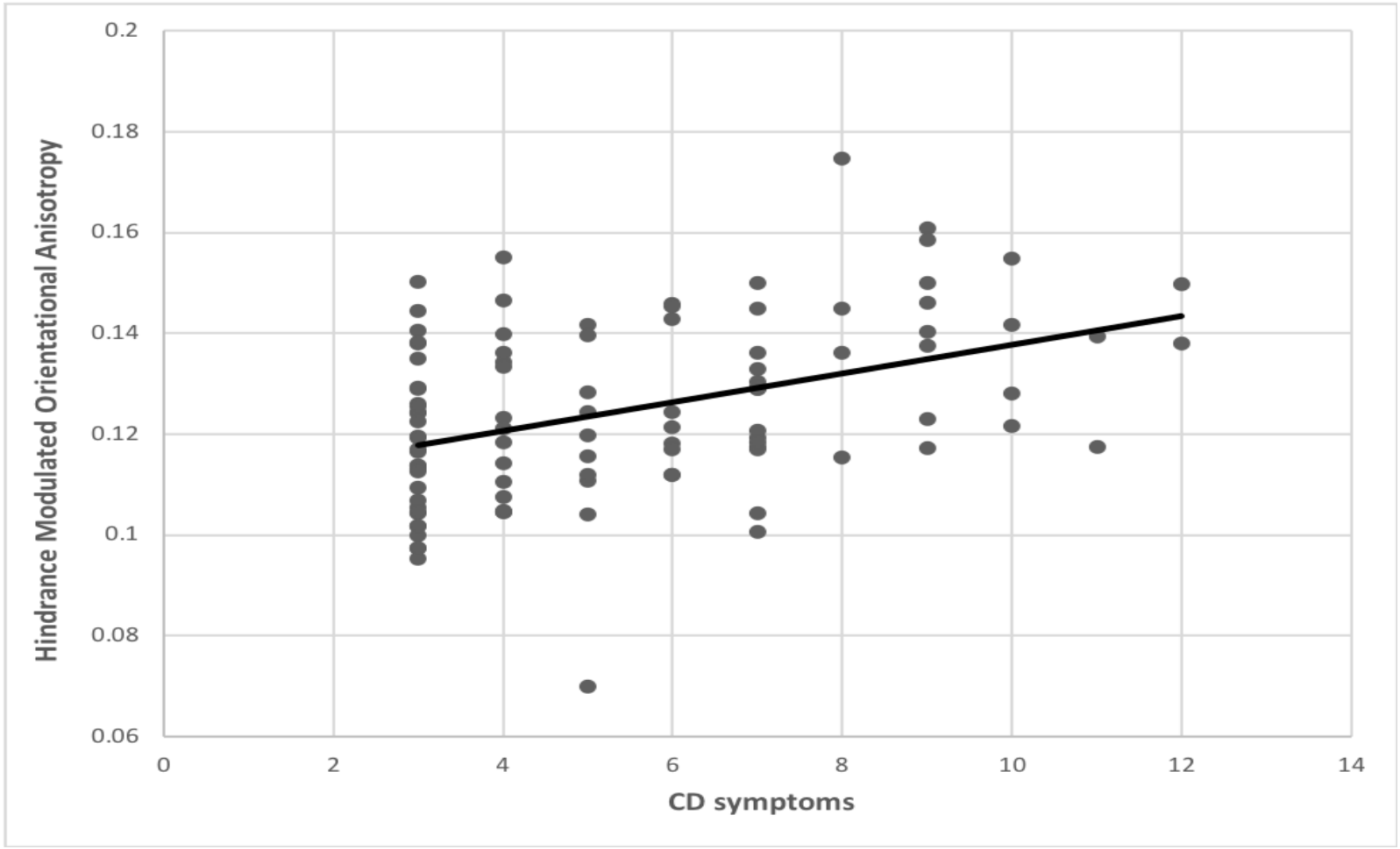

Figure 4. Association between CD symptoms and hindrance modulated orientational anisotropy (HMOA) in the right retrosplenial cingulum in the CD group. There was a significant positive correlation between CD symptoms and HMOA values in the right retrosplenial cingulum tract.