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Evaluating effects of sex and age on white matter microstructural alterations in alcohol use disorder: A diffusion tensor imaging study

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Abstract

Background.—The alterations in white matter microstructure associated with chronic alcohol use have been demonstrated by previous diffusion tensor imaging (DTI) research. However, there is conflicting evidence as to whether such differences are influenced by an individual's biological sex. The purpose of the present study was to investigate the prevalence of sex differences in the white matter microstructure of the brains of individuals with alcohol use disorder (AUD) and healthy controls.

Methods.—One hundred participants with AUD (38 female, ages 21–68) partaking in the NIAAA's inpatient treatment program and 98 healthy control participants (52 female) underwent a diffusion-weighted scan. Images collected were processed for each subject individually and voxelwise tract-based spatial statistics (TBSS) analysis was conducted to measure differences in the DTI measures of fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD).

Results.—A 2-way between-subjects ANCOVA testing for differences in group and sex revealed widespread differences between AUD and control subjects, but no interaction between group and sex. Additional analyses exploring demographic and alcohol use variables showed significant impacts of age on white matter microstructure that were more pronounced in those with AUD. Plots of FA by age and group(s) in major white matter tracts may suggest a future need to explore higher order interactions in larger samples.

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Conflicts of Interest

The authors report no conflicts of interest.

Conclusions.—These results bolster recent findings of similar microstructural properties in men and women with AUD but provide further rationale for the consideration of age when investigating impacts of chronic alcohol use on the brain's white matter.

Keywords

alcohol; alcohol use disorder (AUD); diffusion tensor imaging (DTI); magnetic resonance imaging (MRI); sex differences

1. Introduction

Overarching evidence demonstrates changes in the brain's gray and white matter as a result of the chronic alcohol consumption central to alcohol use disorder (AUD). White matter consists of myelinated axon pathways that connect gray matter regions to each other. Reviews of structural studies have reported deficits in regions including the corpus callosum, striatum, prefrontal cortex, and cingulate cortex, among other areas, influencing a wide range of functions from emotion and cognition to memory (Monnig et al., 2013, Xiao et al., 2015, Yang et al., 2016). In addition to volumetric deficits, diffusion tensor imaging (DTI) studies of the microstructural properties of white matter have revealed compromised integrity in those with AUD compared to controls (Pfefferbaum et al., 2009, Crespi et al., 2020, Sawyer et al., 2018, Fortier et al., 2014). While previous studies consistently demonstrate widespread brain alterations presumably due to excessive alcohol intake, investigations of sex-based susceptibility to alcohol-related changes are mixed and warrant further exploration. An understanding of sex differences in the effects of alcohol use on the brain is useful to guide attention to potential influences of sex in future research as well as to inform more individualized treatment options.

1.1 Macrostructural Sex Differences

Chronic alcohol use significantly impairs the brain, but it remains inconclusive whether alcohol has a similar effect on the brains of men and women. Some have speculated that women's brains may be more susceptible to the effects of alcohol, theorizing that while women tend to show later ages of onset of AUD, alcohol dependence occurs more rapidly, as does accompanying brain atrophy (Pfefferbaum et al., 2001, Mann et al., 2005). This is consistent with studies showing signs of greater damage in females with AUD compared to males with AUD (Hommer et al., 1996, Hommer et al., 2001, Pfefferbaum et al., 2009, Monnig et al., 2015). Such findings include observations of larger brain volume differences between AUD and control women than between AUD and control men (Hommer et al., 2001) as well as greater sensitivity to effects of heavy drinking years in frontal and temporal white matter areas in women with AUD compared to men with AUD (Ruiz et al., 2013). However, others have observed smaller gray and white matter volumes among men with AUD than women with AUD when compared to controls (Pfefferbaum et al., 2001), with one study finding larger reward network volumes in women with AUD compared to controls (Sawyer et al., 2017). Similarly, while one study found greater alterations in the corpus callosum in women with AUD compared to men with AUD and controls (Hommer et al., 1996), another observed more changes to the corpus callosum in men with AUD than females with AUD when compared to controls (Ruiz et al., 2013). Such inconsistencies

between findings suggest the need for further evaluation of the discrepancies related to sex-specific alterations in AUD.

1.2 Diffusion Tensor Imaging (DTI)

Discrepancies in the evaluation of alcohol-induced, sex-specific brain alterations may be due to drinking variation or the inability to establish noticeable changes at the volumetric level. A recent meta-analysis of sex differences in AUD documented the many mixed findings in macrostructural studies, but acknowledged the need for further investigation of these microstructural changes, citing only two studies that have focused on sex differences in AUD using DTI (Verplaetse et al., 2020). DTI is a structural MRI technique that is thought to assess white matter microstructure by measuring the diffusion of water molecules in brain tissue (Soares et al., 2013, Alexander et al., 2007, Basser et al., 1994). DTI provides distinct metrics that can better localize microstructural white matter abnormalities, such as fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD), which are thought to represent aspects of white matter integrity. Higher FA is thought to reflect greater white matter integrity. Decreases in AD are commonly interpreted as a sign of axonal injury, and increases in RD is thought to indicate demyelination of the axon (Alexander et al., 2007, Budde et al., 2009, Beaulieu, 2002, Yeh et al., 2009)

1.3 Microstructural Sex Differences

Previous DTI research identified microstructural properties of compromised white matter within heavy drinking populations that was not apparent through macrostructural volume studies (Pfefferbaum and Sullivan, 2002). The literature points to widespread bilateral deficits in white matter fiber tracts among areas responsible for reward, emotional, cognitive, and other executive functioning processes. Such implicated areas include the body of the corpus callosum, fornix, external capsule, cingulum, superior longitudinal fasciculus, anterior commissure, and inferior frontooccipital fasciculus, among others (Pfefferbaum et al., 2009, Durkee et al., 2013, Yeh et al., 2009, Monnig et al., 2015). Few studies have investigated sex differences in white matter microstructure in AUD populations, and findings have been mixed. Studies suggesting that women may be more susceptible to white matter alterations have demonstrated differences in brain regions including the internal capsule, inferior cingulate, superior longitudinal fasciculus, cerebellar hemisphere, pontocerebellar bundles, and left fusiform gyrus (Pfefferbaum et al., 2009, Fortier et al., 2014). Additionally, Monnig et al. (2015) found that heavy-drinking females exhibited a significant relationship between alcohol consumption and white matter alterations that was not present in matched male subjects; however, they also observed significantly lower FA in women across groups. Most recent investigations have failed to find evidence supporting a more profound effect of alcohol on the female brain. Findings from Sawyer et al. (2018) using a whole-brain approach showed that men with AUD had lower FA than control men, but women with AUD had higher FA than control women. Similar results were found in an evaluation of the medial forebrain bundle, where men with AUD demonstrated lower FA and higher RD compared to controls, but women with AUD showed higher FA and lower RD (Rivas-Grajales et al., 2018). Such inconsistencies could be contributed to differences in treatment-seeking status, levels of alcohol consumption, polysubstance use, comorbidities, or differences in DTI parameters among other variables. However, these findings still highlight the inconclusive

nature of sex differences in AUD measured by DTI and suggest a need for further research exploring sex-specific differences in the effects of alcohol on white matter microstructure.

1.4 The Current Study

This study was designed to identify sex differences in white matter microstructure in abstinent individuals with AUD and healthy controls, attempting to bring clarity to the inconsistent literature through the use of a relatively large sample size that allowed for a whole-brain approach and formal test of the interaction between sex and group status. A whole-brain approach was used given previous evidence for changes in FA across various brain regions, lacking consistency in specific region(s) of interest. As structural differences have been detected in studies of white matter microstructure where they were not seen in macrostructure (Pfefferbaum and Sullivan, 2002), the current study selected to investigate microstructural properties alone in an attempt to better understand these potentially more nuanced differences. The current study sought to i) confirm the previous findings of widespread differences in microstructural white matter properties in those with AUD compared to controls, ii) test for a significant interaction between AUD status and sex, iii) and explore the relationship between white matter microstructural properties and demographic or alcohol consumption variables that may explain discrepancies in the previous literature.

We hypothesized (h_i) that there would be widespread differences between a group of treatment-seeking subjects with AUD (AUD) and a group of healthy control (CON) participants, where AUD participants would have lower FA, lower AD, and higher RD when compared to CON participants. Additionally, based on the majority of findings from microstructural studies, we expected (h_{ii}) to observe a significant interaction between sex and AUD group status on white matter microstructure. Specifically, we hypothesized that additional contrasts including females with AUD (AF), males with AUD (AM), control females (CF), and control males (CM) would reveal lower FA, lower AD, and higher RD in females with AUD compared to males with AUD, indicating greater white matter microstructural differences in females.. However, given that some recent studies have failed to find these differences, we further explored the relationship between DTI measures of white matter fibers with various demographic and alcohol use variables. This portion of the analysis was exploratory; as such, we had no formal hypotheses for aim iii.

2. Methods and Materials

2.1 Participants

Subjects consisted of 198 right-handed individuals, ages 21–68, enrolled in the National Institute on Alcohol Abuse and Alcoholism (NIAAA)'s screening protocol either as healthy controls or inpatients receiving treatment for AUD. Participants in the AUD group (n=100, 38 F, 25–68 years old) were individuals partaking in NIAAA's inpatient treatment program at the NIH clinical center between 2014 and 2020 and were abstinent at the time of the scan (minimum = 6 days, maximum = 44 days, see Table 1). The NIAAA's treatment program includes group and individual therapy as well as pharmacological intervention when appropriate. Since the language defining substance use disorders shifted during this

time, all AUD participants either met criteria for alcohol dependence (based on DSM-IV criteria) or moderate to severe AUD (based on DSM-5 criteria; Compton et al., 2013). Healthy control participants (n=98, 52 F, 21–65 years old) were enrolled in studies through the NIAAA outpatient clinic at the NIH clinical center in the same time frame. None of the control participants met criteria for alcohol abuse or dependence (based on DSM-IV criteria) or for AUD (based on DSM-5 criteria).

Both AUD and CON groups participated in screening protocols in which they completed the Alcohol Time-Line Followback (TLFB) (Sobell et al., 1996), Fagerstrom Test for Nicotine Dependence (Heatherton et al., 1991), Lifetime Drinking History (LDH) (Skinner and Sheu, 1982), Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993) and the Structured Clinical Interview for DSM-IV Axis 1 Disorders (SCID-IV) (First et al., 2007) or Structured Clinical Interview for DSM-5 Disorders (SCID-5-RV) (First et al., 2015) (see Table 1). The presence of comorbid diagnoses is described in Table 2, combining DSM-IV and DSM-5 terminology. The LDH, which provides an estimate of drinking patterns across an individual's life, was used to quantify heavy drinking years, defined as periods of time in which individuals drank more than 6 drinks per day, in accordance with the LDH Manual.

Exclusion criteria included neurological disorders, history of significant head trauma, claustrophobia, non-removable ferrous metal in the body, positive urine drug tests on the day of imaging, positive pregnancy tests (if applicable), and active withdrawal symptoms as determined by the Clinical Institute Withdrawal Assessment (CIWA ≥ 8). Imaging data was collected as part of a larger NIAAA protocol, which collects both structural and task-based MRI data. Some of the subjects included in this sample have been included in other analyses of task-based fMRI, but the DTI data from these subjects has not been included in any previous publications.

2.2 DTI Acquisition

After providing written informed consent, participants underwent a T1-weighted scan, a fat suppressed T2-weighted scan, and a DWI scan as part of a structural and functional study. All scans were collected on a 3 Tesla MRI scanner (Siemens Skyra) with a 20-channel head coil. The T1-weighted scan was collected using an MPRAGE sequence with 128 axial slices, TR of 1900 ms, TE of 30 ms, 256 × 256 matrix, and 0.94 × 0.94 × 1.0 mm^3 voxel size. The DTI scan contained 80 volumes with TR of 8400 ms, TE of 84.0 ms, 2.5 × 2.5 × 2.5 mm voxel size, and in-plane acceleration factor of 2. Of the 80 volumes collected, 10 were collected at b value of 0 s/mm^2, 10 at b value of 300 s/mm^2, and 60 at b value of 1100 s/mm^2. A fat suppressed T2-weighted scan with 100 axial slices, TR of 11762.5 ms, TE 90.0 ms, 150 × 192 matrix, and 1.7 × 1.7 × 1.7 mm^3 voxel size was collected for use in the post-processing pipeline for EPI distortion correction. EPI distortions have been shown to impact the accuracy of fiber tracking of DTI images, and fat suppressed T2 images have been shown to produce better results than T1 images to correct for such distortions (Irfanoglu et al., 2012). All images were visually inspected prior to data processing.

2.3 DTI Preprocessing

Each participant's DTI data was preprocessed individually using the TORTOISE software package (Pierpaoli et al., 2010) (https://tortoise.nibib.nih.gov/). As recommended in the software, each DTI volume was individually viewed for quality assessment. Volumes that contained slice dropout due to subject motion were removed from the data set. Participants with greater than 10% of total volumes (8 volumes) corrupted were excluded from the analysis and are not included in the provided sample size. The data then underwent DIFF_PREP, part of TORTOISE software (Pierpaoli et al., 2010), to correct for motion, eddy current distortions, EPI related distortion, and reorient the diffusion-weighted images and corresponding b-matrix into a target space. The target space was each subject's T1-weighted image. DIFF_CALC was then used to estimate the diffusion tensors (DTs) along with their parameters.

2.4 Tract-Based Spatial Statistics (TBSS) and Analysis

After obtaining a final FA image for each subject, voxelwise statistical analysis was conducted using TBSS (Smith et al., 2006) as a part of the FSL toolbox (Smith et al., 2004). FA images were aligned into a 1 × 1 × 1 mm standard space, using the recommended Montreal Neurological Institute (MNI) template. Then, the nonlinear transforms were brought into standard space to create a mean FA image and a mean FA skeleton, which represented tracts common to all participants at a threshold of 0.2. Voxelwise cross-subject statistics for FA, AD, and RD were applied using FSL's randomise (Winkler et al., 2014) for nonparametric permutation testing. In order to tests hypotheses *i* and *ii*, we constructed a generalized linear model (GLM) to assess the main effect of group (AUD vs. CON), main effect of sex (male vs. female), and the interaction between group and sex, using 5000 permutations. The resulting 2-way between-subjects ANCOVA included a 0.05 significance threshold and corrected for multiple comparisons using the threshold-free cluster enhancement (TFCE) option within randomise. Age and level of education were included in the GLM as a covariates. Other variables, such as alcohol consumption, drinking years, smoking status, and comorbid diagnoses, could not be included as covariates since they occurred almost solely in the AUD group. To further evaluate the directionality of our hypotheses *i* and *ii*, contrasts were constructed within the ANCOVA analysis to compare subgroups, including: AF vs. AM, AF vs. CF, AM vs. CM, and CF vs. CM. All of the above comparisons were run within the single model that was constructed using randomise and thus were corrected for by the TFCE option. Locations of significant clusters were determined using the autoaq command within FSL, which estimates the probability of each cluster belonging to a specific WM tract based on the JHU White Matter Tractography Atlas (Hua et al., 2008). We used an arbitrary cutoff of 50 voxels when reporting significant clusters to avoid small clusters and further restrict false positive voxels.

No measures of alcohol use were included in the primary analysis described above, as they are presumed to confound the effect of group. However, in exploratory follow-up analysis within the AUD group only, we did assess their relation to FA, AD, and RD scores using a correlational approach. After completing TBSS, we created binary masks of significant clusters in the main effect of group (AUD vs. CON) to extract mean values for each subject. These mean scores for subjects in the AUD group were then correlated with

demographic and alcohol use variables, including age, level of education, number of drinks in the 30 days preceding admission, heavy drinking years, age of first drink, days since last drink, and AUD severity. To further understand the effects of these variables on white matter microstructural properties, we also ran partial correlations that controlled for age. All correlations were run using Pearson's correlations.

Given the strong influence of age on FA within the correlational analysis and the previous literature establishing that FA declines with age (Salat et al., 2005, Burzynska et al., 2010), we felt it would be informative to examine the interaction between age and AUD status on whole brain FA in a GLM using FSL's randomise in the same methods described for the primary analysis above (5000 permutations, a 0.05 significance threshold, and threshold free cluster enhancement (TFCE) to correct for multiple comparisons within the model). The model also included level of education as a covariate. It should be emphasized that this analysis was not planned *a priori* and was run to further investigate age effects within our sample after obtaining correlational results; thus, the risk of false positives in this analysis are inflated. Given the significant age difference between AUD and CON groups, we also reran the same primary ANCOVA analysis on a subset of age- and sex-matched participants (n(AUD)=60, n(CON)=60). Male and female AUD participants were also matched based on heavy drinking years and drinks consumed in the 30 days prior to admission (see Table S1).

3. Results

3.1 Fractional Anisotropy

The ANCOVA analysis of differences in FA revealed two significant clusters across white matter tracts when testing the main effect of group (CON>AUD). CON showed significantly greater FA in the anterior thalamic radiation, corticospinal tract, cingulum, forceps major and minor, inferior frontooccipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus, and uncinate fasciculus compared to AUD (see Table 3, Figure 1A). There was a relatively small significant cluster of bilateral differences in FA between CM and CF (CM>CF) including the anterior thalamic radiation and corticospinal tract. As expected, we observed significant effects of group within the male and female groups individually. Both the CM and CF groups showed significantly greater FA than AM and AF groups, respectively, in the same regions observed when testing the main effect of group (see Table 3, Figure 1C and D). The tests of both the main effect of sex and the interaction between group and sex showed no significant results, and we did not observe any significant difference between AM and AF groups. As outlined above, the primary analysis accounted for age and level of education, but not differences in alcohol consumption. An analysis of subjects matched on sex, age, and alcohol consumption (n(AUD) = 60, n(CON) = 60) revealed similar widespread group differences, no main effect of sex, and no interaction between group and sex.

3.2 Axial and Radial Diffusivities

When further investigating individual properties of white matter microstructure, we saw significantly greater AD in the AUD group in the anterior thalamic radiation compared to the CON group. Additionally, the AUD group demonstrated significantly greater RD in the same

regions as described in the FA results, except the forceps minor (see Table S2, Figure S1). In the test of the main effect of sex, male subjects showed significantly greater AD in all of the above-mentioned regions, except the forceps minor and uncinate fasciculus (See Table S3, Figure S2). This apparent difference in AD between males and females no longer appeared when subjects were matched based on age and alcohol consumption and may presumably be contributed to greater alcohol use in the male sample. We did not observe a significant interaction between group and sex, nor any differences between the AF and AM groups when investigating differences in AD and RD.

3.3 Correlations with Demographic and Alcohol Use Variables

Within the AUD group, correlations of demographic and alcohol use variables with diffusivity measures of the significant clusters of group differences (AUD vs. CON) showed a relationship between age and heavy drinking years with FA, AD, and RD values. Additionally, AUDIT scores were significantly positively correlated with AD values, but not FA or RD values. Both age and heavy drinking years, which unsurprisingly showed significant positive correlations with each other, correlated negatively with FA values and positively with AD and RD values (see Table S4). We no longer observed a significant relationship between heavy drinking years and DTI measures when conducting partial correlations that controlled for age.

3.4 Interaction between Age and Group Status

To further explore impacts of age on white matter microstructure in conjunction with potential impacts of AUD, we ran a generalized linear model of the interaction between age and group status (AUD vs. CON). These analyses were run in both the primary and age-matched samples using subject's FA images. Comparison to the JHU White Matter Atlas revealed significant effects in the anterior thalamic radiation, corticospinal tract, cingulum, forceps major and minor, inferior frontooccipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus, and uncinate fasciculus in both analyses. Significant regions were more widespread in the matched analysis (see Figure S3) than in the primary analysis (see Figure 2).

To aid interpretation of these findings, we plotted each subject's mean FA from each major white matter tract by age and AUD status (see Figure 3, Figure S5). These plots suggest steeper age-related decline in FA in individuals with AUD in most regions, with the exception of the left cingulum and left corticospinal tracts and with minimal observable differences in the uncinate fasciculus. In line with our original aim and for the sake of completeness, we further broke down the plots by sex. Visual patterns in certain tracts may suggest a complex sex-differential association between age, AUD, and FA, particularly in the left cingulum and right uncinate fasciculus, though these differences are slight and the three-way interaction was not formally tested.

4. Discussion

4.1 Group Differences

Consistent with previous studies, we observed differences between AUD and CON groups in terms of FA and RD, with the AUD group demonstrating lower FA and higher RD. Areas showing significant differences were widespread, affecting areas within the frontal, temporal, parietal, and occipital lobes of the brain. Though our current study is not designed to infer causality, this may suggest potential alcohol-related damage to the myelination of the anterior thalamic radiation, cingulum, fronto-occipital fasciculus, etc., which collectively, indicate implications for visual, motor, attentional, emotional, and working memory processes (Coenen et al., 2012, Bubb et al., 2018, Wu et al., 2016, Herbet et al., 2018, Wang et al., 2016, Von Der Heide et al., 2013). Findings of differences in these areas also align with previous findings of compromised networks implicated in addiction and involved in reward processing and executive functioning (Goldstein and Volkow, 2011, Monnig et al., 2015, Pfefferbaum et al., 2009, Koob and Volkow, 2010, Yeh et al., 2009).

While the underlying properties reflected in diffusivity measures are still controversial, they are generally thought to reflect axonal diameter and integrity, myelination, and crossing fibers (Wheeler-Kingshott and Cercignani, 2009, Bennett et al., 2010). Traditional interpretations, derived largely from animal studies, postulate lower AD as reflective of axonal injury, which led us to hypothesize lower AD in the AUD group compared to the CON group (Yeh et al., 2009, Winklewski et al., 2018). Upon further review, our finding of higher AD in the AUD group is consistent with prior studies of chronic alcohol use (Pfefferbaum et al., 2014, Topiwala et al., 2017, Crespi et al., 2020). In the context of heavy drinking, Yeh and colleagues (Yeh et al., 2009) postulate that increased AD in abstinent treatment-seekers could be due either to the intersection of white matter fibers in some regions or to the presence of debris-removing proteins facilitating axonal repair. Changes in axon diameter due to potential impacts of dehydration on the brain could also potentially explain increased AD in heavy drinking populations. However, it seems most likely that these findings can be contributed to inaccuracies in diffusivity measures that occur in cases where white matter is severely damaged (Winklewski et al., 2018, Wheeler-Kingshott and Cercignani, 2009). A recent review by Winklewski and colleagues discusses these cases of increased AD across pathologies where damage to the axon is expected. With knowledge gained largely through studies on multiple sclerosis, they conclude that axial diffusivity is not a reliable measure of axonal injury in cases of severe white matter microstructural impairments, in particular when inflammation, demyelination, axonal loss, and axonal injury may co-occur (Winklewski et al., 2018). Given the established impacts of chronic alcohol use on inflammation and changes in white matter (Monnig et al., 2013, Fortier et al., 2014, Crespi et al., 2020, de la Monte and Kril, 2014), it may not be reasonable to consider axial diffusivity in future studies of AUD populations.

4.2 Sex Differences

We found no evidence for an interaction between sex and AUD status, our formal test for sex differences, in alcohol-related microstructural alterations in our ANCOVA model. We did observe significant differences between males and females in the primary analysis,

without respect to group, primarily in terms of AD. Control males demonstrated higher FA in the anterior thalamic radiation and corticospinal tract compared to control females. Studies of healthy volunteers have shown structural white matter differences, with males generally having greater white matter volume and FA compared to females (Ritchie et al., 2018, Ingalhalikar et al., 2014), although this may be accounted for by overall differences in intracranial volume (Takao et al., 2014). There were widespread differences in AD between males and females, and between the CM and CF groups, with males showing greater AD than females. These sex differences did not appear in the age-matched groups, and it seems most likely that the observed sex differences in AD in the primary sample may be due to greater alcohol use in males compared to females. With the potential for these differences to be related to differences in intracranial volumes, we assessed the correlations between estimated total intracranial volumes (eTIV) and DTI measures. These correlations revealed a significant relationship between eTIV with FA1, AD1, and RD1, which were the largest clusters of group differences in the primary analysis (see Table S5). The strongest relationship occurred between eTIV with AD, which could help explain our observation of greater AD in males in the primary sample, as eTIV tends to be larger in men than in women. As previously discussed, recent studies have also failed to demonstrate sex differences at the microstructural level. Our findings add to this relatively small pool of studies that have evaluated sex-specific microstructural alterations in AUD, continuing to refute the previous theory that female brains are more susceptible to alcohol-related changes than male brains (Sawyer et al., 2018, Fortier et al., 2014, Rivas-Grajales et al., 2018).

4.3 Relationships with Demographic and Alcohol Use Variables

The exploratory results of the partial correlations suggested that age was the primary factor impacting white matter microstructure within the groups of this sample, and correspondingly, that heavy drinking years had little relationship to microstructural alterations. The key impact of age was not surprising, although we also anticipated an additive effect on microstructural properties with greater alcohol use, given that previous studies have found significant effects of greater alcohol use within heavy drinking samples. For example, a study by Fortier et al. (2014) found significant or trend level negative correlations between alcohol use variables including average drinks per day, heavy drinking years, and alcohol severity with FA in the inferior frontal gyrus. However, the significant interaction between age and AUD group status we detected (see Figure 2) does suggest that alcohol consumption still plays a role in this effect, consistent with the literature. As previously mentioned, FA is known to decrease with age (Burzynska et al., 2010, Salat et al., 2005), an effect that Pfefferbaum et al. (2001) and Pfefferbaum et al. (2014) found to be more pronounced in chronic alcohol users. While the tracts with significant age by AUD group interactions are the same as those implicated in the main group effects, we do not believe age differences drive the main effects. The main effects were substantively the same in age-matched subgroups, and visual comparison of the main effects with the age and group interaction effects (see Figure 1A, Figure 2) shows that significant areas within the tracts differ slightly. Notably, it appears that the group effect revealed differences in more of the corpus callosum, where the interaction between age and group status appeared to have an effect on the corpus callosum primarily in the frontal region of the brain.

The plots of FA by age and group in each major white matter tract allowed for visualization of the interaction effect between AUD status and age, in which the AUD group demonstrated a steeper decline in FA in most regions. The left cingulum and right uncinate fasciculus emerged as areas of potential interest for further investigation when plotting age and FA by subgroups (AF, AM, CF, CM). While controls in these regions showed similar patterns regardless of sex, individuals in the AUD group showed a slight potential for a sex-differential effect of age on FA. These observations require formal testing in order to provide any meaningful clarification to the potential influence of sex on the interaction of age and AUD. With regards to these particular regions, the cingulum has been associated with inhibitory control, motivation, and working memory (Koob and Volkow, 2010, Schmahmann et al., 2007, Bubb et al., 2018), while the uncinate fasciculus is thought to be involved in learning, memory, and reward processes (Von Der Heide et al., 2013). Further studies should formally test the presence of an interaction between age, sex, and AUD status, as this was not done in the present study due to the exploratory nature of age-related investigations. Additionally, further investigations may choose to include measures of cognitive, emotional, and decision making processes associated with areas such as the cingulum and uncinate fasciculus, which may inform whether cognitive disruptions and reward-seeking behaviors associated with AUD differ between men and women.

4.4 Limitations

The potential for crossing fibers serves as a recognized limitation to the interpretability of DTI measures. Crossing fibers carry the greatest implications for the interpretation of AD and RD by making it difficult to assume the directions of the axons to determine whether water is moving in the preferential direction of diffusion (Wheeler-Kingshott and Cercignani, 2009). As discussed above, measures of AD and RD may also be particularly inaccurate in the co-occurring presence of inflammation, demyelination, and axonal injury (Winklewski et al., 2018). In addition, our findings are limited by a cross-sectional design, with only one scan for each subject. As a result, we cannot definitively conclude the impacts of alcohol on the brain structure of individuals in the AUD group. All alcohol use data is based on retrospective self-report, which could lead to misreporting, especially given that exact quantities of alcohol in heavier drinking periods may be difficult to recall.

Subjects in the AUD group had significantly fewer years of education than controls, which was covaried in all analyses; however, they also presented with greater comorbid diagnoses and tobacco smoking. These variables were not controlled for as they occurred almost solely within the AUD group and could not be effectively parsed out from the effects of group within this sample. The prevalence of tobacco smoking in our sample is not surprising as an estimated 80% of individuals who meet DSM-IV criteria for alcohol dependence also report smoking cigarettes (Castillo-Carniglia et al., 2019). The effects of polysubstance use on brain structure remain inconclusive, with one study showing similar or greater gray and white matter volumes in those with comorbid substance use disorders (Mon et al., 2014) but another showing greater structural impairments in those who use more substances (Kaag et al., 2017). Similarly, one study found a significant relationship between smoking history and lower FA in AUD (Zou et al., 2017), while another observed no relationship (Monnig et al., 2015). The inability to control for these factors in the present study serves as a

major limitation, yet the literature surrounding polysubstance use is not conclusive enough to indicate how much smoking status and comorbidities may impact white matter properties in our sample.

AUD has been shown to be highly comorbid with other psychiatric disorders with up to 40% of individuals with a diagnosis of major depression disorder (MDD) in the U.S. also meeting criteria for AUD, up to 40% of those with an anxiety disorder having a comorbid AUD diagnosis, and up to 55% of those with post-traumatic stress disorder (PTSD) also meeting criteria for AUD (Castillo-Carniglia et al., 2019). While differences have been inconsistently observed, MDD has most often been associated with changes in the white matter microstructure of the corpus callosum, superior longitudinal fasciculus, arcuate fasciculus, and in the right cerebellar hemispheric lobule (Chen et al., 2016, Jiang et al., 2017). DTI studies of anxiety disorders and PTSD have yielded inconsistent results; however, some studies have indicated differences in white matter microstructure in the uncinate fasciculus in those with an anxiety disorder and in the cingulum in those with PTSD (Tromp et al., 2012, Madonna et al., 2019, Prasad et al., 2019). Additionally, our group has previously conducted a study, using a different sample, in which we found no significant differences between cases of AUD with and without comorbid PTSD using DTI (Durkee et al., 2013). While the presence of comorbidities in our sample may influence observed differences and prevents us from drawing causal conclusions, it is representative of the population of individuals with AUD.

This study investigates differences in terms of binary biological sex. We did not investigate sex hormones or other biological variables that would confirm the specific role of biological sex, so it is possible that our results are explained by gender identity rather than biological sex. Additionally, we did not collect measures sensitive to variation in gender identity that would have allowed us to rule out the potential impact of identity rather than biology. While this study aligns with the framework of previous research in using biological sex in the investigation of sex differences, we recognize that failing to include measures of gender identity limits the generalizability of our findings.

Although our sample was moderately sized (N = 198), our power was relatively limited; it is therefore possible that our failure to detect an interaction of sex and AUD on white matter microstructure may not reflect a true negative. As previously discussed, age had a large impact on the findings and interacted significantly with AUD; age may have washed out a relatively small effect of sex. It is also possible that a complex higher order interaction between age, AUD, and sex may underly the observed patterns; indeed, plots in several tracts hint at this possibility. However, this study is underpowered to detect such an effect, or to parse out the true impact of other measured and unmeasured comorbidities. Future studies should leverage large, multisite data collection protocols to address these concerns.

5. Conclusion

Overall analyses revealed significant effects of AUD on white matter microstructure, regardless of an individual’s sex. Significant differences between AUD and CON were widespread, impacting regions previously implicated in addiction. We found no formal

evidence for greater alcohol-related differences in females compared to males, falling in line with more recent studies finding a lack of sex-specific white matter alterations in AUD. Further analysis revealed that age seemed to overwhelm most effects of alcohol use variables, such as heavy drinking years and average drinks per day, on white matter fiber integrity. However, investigating the separate roles of age and severity of alcohol use on white matter microstructure requires properly designed longitudinal studies. This addition to the current mixed literature around alcohol-related sex differences calls for further exploration of various factors that may lie behind contradicting findings in sex-specific brain alterations. Future investigations may benefit from region-specific approaches, inclusion of measures of cognitive and reward processes, and consideration of more pronounced age effects in individuals with AUD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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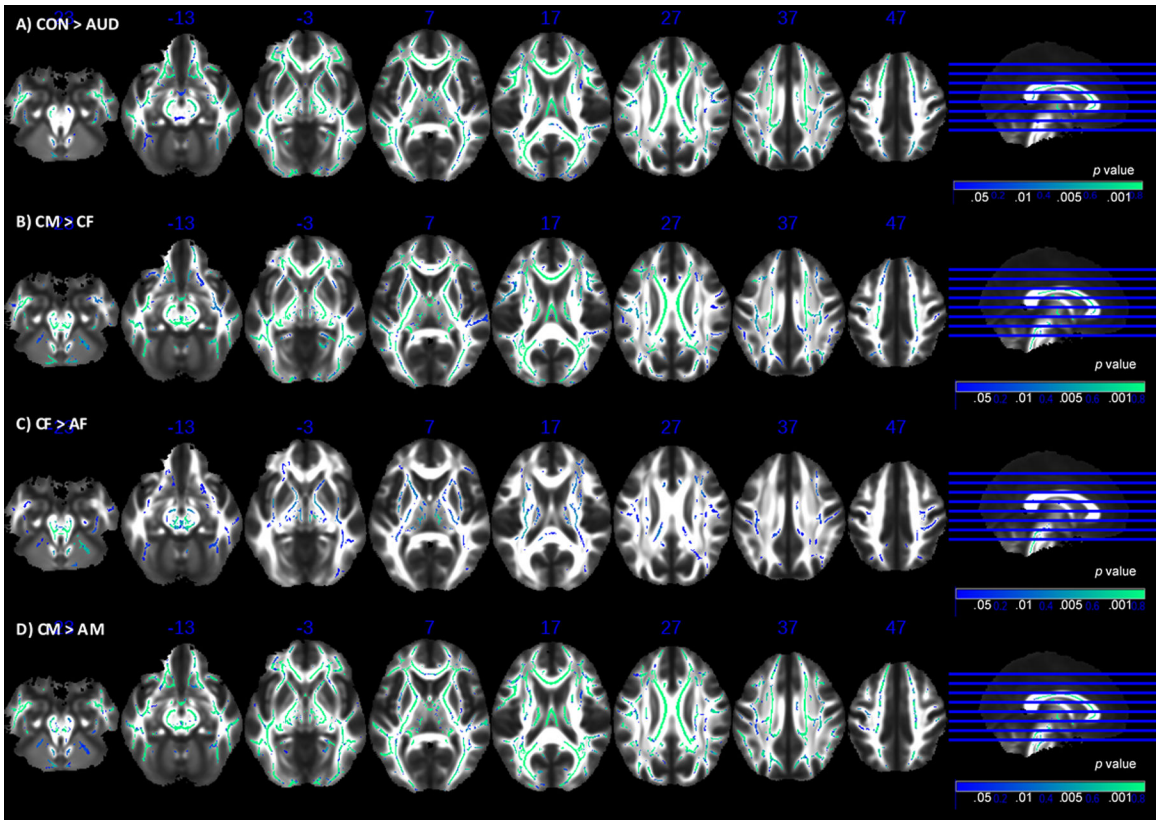

Figure 1. Significant Clusters for Differences in FA, All Subjects (N = 198)

Results reflect findings from the primary analysis. Difference in colors represents level of significance as demonstrated by the sliding scale for *p* found at the bottom right corner of each image. Displayed slices were selected after visually inspecting the whole brain to show all significantly affected areas. Significant clusters are overlayed on the mean FA image created from all subjects FA images as part of the TBSS pipeline. FA = fractional anisotropy, AUD = individuals with alcohol use disorder, CON = healthy controls, AF = females with AUD, AM = males with AUD, CF = female controls, CM = male controls

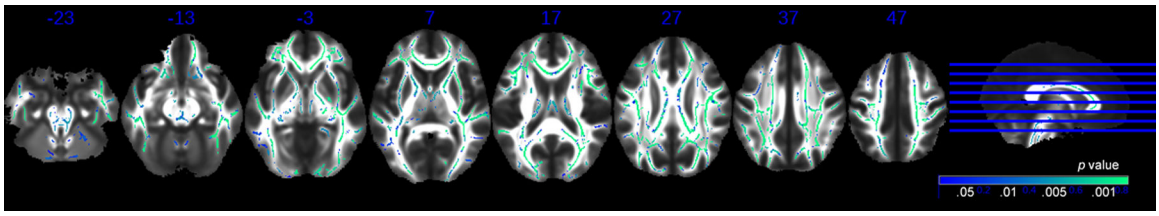

Figure 2. Interaction Between Age and Group (AUD vs. CON) in the Primary Analysis (N = 198)

Results reflect findings from the primary analysis. Difference in colors represents level of significance as demonstrated by the sliding scale for *p* found at the bottom right corner of each image. Displayed slices were selected after visually inspecting the whole brain to show all significantly affected areas. Significant clusters are overlayed on the mean FA image created from all subjects FA images as part of the TBSS pipeline.

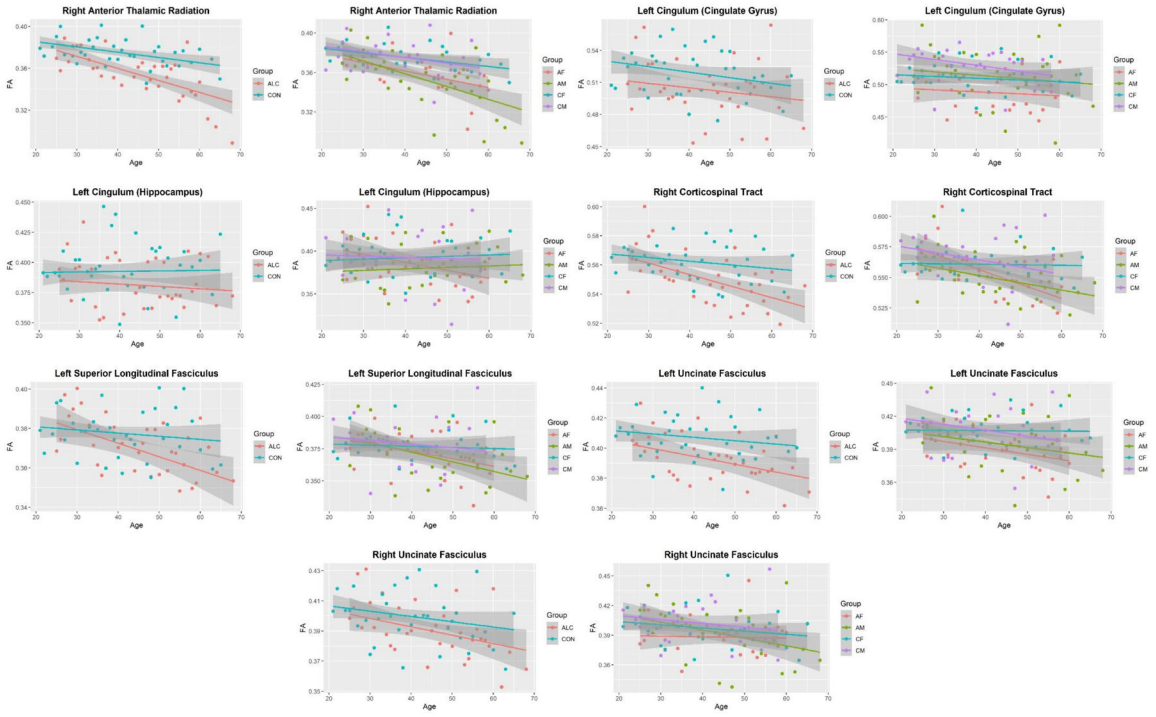

Figure 3. FA Plotted by Age and Group(s) (AUD vs. CON) (AF, AM, CF, CM) in the Primary Analysis (N = 198)

Binary masks of each major white matter tract identified by the JHU White Matter Tractography Atlas (MNI space) were multiplied by each subject's standardized FA image (also in MNI space) to obtain mean FA values for individual subjects for each major tract. Subject's mean FA scores in these areas are plotted by their age and group status. Subgroups (AF, AM, CF, CM) allow for visualizations of FA across age, AUD group, and sex. Graphs of all regions are shown in Figure S5. The left anterior thalamic radiation (ATR) showed similar patterns to the right ATR, forceps major and minor, left and right inferior longitudinal fasciculus (ILF), left and right inferior fronto-occipital fasciculus (IFOF). The left superior longitudinal fasciculus (SLF) showed similar patterns to the rest of the SLF. FA = fractional anisotropy, AF = females with AUD, AM = males with AUD, CF = female controls, CM = male controls

Table 1.

Demographics of Primary Study Sample (N = 198)

	AF (n=38)	AM (n=62)	Statistics between AF and AM	CF (n=52)	CM (n=46)	Statistics between CF and CM	Statistics between AUD and CON
Age M(SD) (Min – Max)	43.24 10.20) (25 – 60)	43.58(11.35) (25 – 68)	$t = 0.16$ $p = 0.88$	37.00(11.94) (21 – 65)	36.80(10.12) (21 – 58)	$t = -0.09$ $p = 0.93$	$t = -4.19$ * $p < .001$
Years of Education M(SD)	13.95 (2.04)	13.58 (2.71)	$t = -0.77$ $p = 0.44$	16.33 (3.07)	16.91 (3.55)	$t = 0.87$ $p = 0.39$	$t = 6.95$ * $p < .001$
Drinks Past 30 Days M(SD)	289.50 (174.62)	418.51 (282.59)	$t = 2.82$ * $p = 0.006$	7.66 (10.80)	14.25 (16.74)	$t = 2.28$ * $p = 0.03$	$t = -14.10$ * $p < .001$
Heavy Drinking Years M(SD) (Min – Max)	12.84 (9.39) *(0 – 38)*	17.35(11.12) *(0 – 43.7)*	$t = 2.02$ * $p = 0.047$	---	---	---	---
Days since Last Drink M(SD)	21.95 (8.63)	22.51 (7.77)	$t = 0.33$ $p = 0.745$	---	---	---	---
AUDIT M(SD)	28.75 (6.11)	28.28 (5.63)	$t = -0.33$ $p = 0.74$	2.31 (1.83)	3.44 (2.78)	$t = 2.29$ * $p = 0.02$	$t = -37.52$ * $p < .001$
Smokers (%)	23 (61)	40 (65)	$X^2 = 0.04$ $P = 0.85$	0	1 (2)	$X^2 = 0.004$ $P = 0.95$	$X^2 = 84.11$ * $p < .001$
Axis 1 Alcohol Dependence / AUD (%)	38 (100)	62 (100)	---	0	0	---	---
Axis 1 besides Alcohol Dependence (%)	26 (68)	37 (60)	$X^2 = 0.39$ $P = 0.53$	2 (4)	0	$X^2 = 0.44$ $P = 0.51$	$X^2 = 80.67$ * $p < .001$

Twenty-eight subjects had incomplete data for the above measures with 14 missing heavy drinking years and 22 missing AUDIT scores.

* indicates $p < .05$,

M = Mean, *SD* = Standard Deviation, AUDIT = Alcohol Use Disorders Identification Test, AUD = individuals with alcohol use disorder, CON = healthy controls, AF = females with AUD, AM = males with AUD, CF = female controls, CM = male controls

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Table 2.

Current Axis-I Disorders from SCID-IV/SCID-5 Interviews in the Primary Study Sample (N = 198)

Axis 1 Diagnosis	AF (n=38)	AM (n=62)	CF (n=52)	CM (n=46)
Substance Use Disorders (%)				
Cannabis Dependence	4 (11)	11 (18)	0	0
Cannabis Abuse	0	1 (2)	0	0
Cocaine/Stimulant Dependence	3 (8)	11 (18)	0	0
Opioid Dependence	0	2 (3)	0	0
Sedative, Hypnotic, or Anxiolytic Dependence	1 (3)	0	0	0
Sedative, Hypnotic, or Anxiolytic Abuse	0	1 (2)	0	0
Hallucinogen Dependence	0	2 (3)	0	0
Other Substance Dependence	0	1 (2)	0	0
Mood/Depressive Disorders (%)				
Major Depressive Disorder	9 (24)	13 (21)	1 (2)	0
Dysthymic/Persistent Depressive Disorder	5 (13)	5 (8)	0	0
Alcohol-Induced Mood/Depressive Disorder	5 (13)	9 (15)	0	0
Other Specified Depressive Disorder	0	1 (2)	0	0
Anxiety Disorders (%)				
Generalized Anxiety Disorder	5 (13)	5 (8)	1 (2)	0
Specific Phobia	2 (5)	0	1 (2)	0
Agoraphobia	1 (3)	0	0	0
Social Phobia/Social Anxiety Disorder	8 (21)	2 (3)	0	0
Anxiety NOS	1 (3)	1 (2)	0	0
Panic Disorder	1 (3)	0	0	0
Alcohol-Induced Anxiety Disorder	1 (3)	2 (3)	0	0
Obsessive-Compulsive Disorder	1 (3)	0	0	0
Trauma Disorders (%)				
Posttraumatic Stress Disorder	13 (34)	2 (3)	0	0
Other Specified Trauma Disorder	1 (3)	2 (3)	0	0
Adjustment Disorder	0	1 (2)	0	0
Bereavement (%)	0	1 (2)	0	0

Axis 1 Diagnosis	AF (n=38)	AM (n=62)	CF (n=52)	CM (n=46)
Attention-Deficit Hyperactivity Disorder (%)	3 (8)	5 (8)	0	0
Bipolar Disorder and Related Disorders (%)				
Bipolar I Disorder	1 (3)	0	0	0
Bipolar II Disorder	0	1 (2)	0	0
Substance-Induced Bipolar Disorder	0	1 (2)	0	0

Reported as raw number of participants who met criteria for each disorder based on SCID-IV/SCID-5. Terminology reflects DSM-IV classification of substance abuse and dependence that DSM-5 combined into substance use disorders.

AUD = individuals with alcohol use disorder, CON = healthy controls, AF = females with AUD, AM = males with AUD, CF = female controls, CM = male controls

Table 3.

Significant FA Clusters in the Primary Analysis (N = 198)

Comparison (Group 1 > Group 2)	Assigned Cluster Number	White Matter Tracts	Voxels	X (mm)	Y (mm)	Z (mm)	Group 1 *M* (*SD*)	Group 2 *M* (*SD*)	*p*
CON > AUD	FA 1	Anterior thalamic radiation R Corticospinal tract L & R Cingulum (cingulate gyrus) L & R Cingulum (hippocampus) L & R Forceps major Forceps minor Inferior fronto-occipital fasciculus L & R Inferior longitudinal fasciculus L & R Superior longitudinal fasciculus L & R Uncinate fasciculus L & R Superior longitudinal fasciculus (temporal part) L & R	30,896	12	29	−10	0.52 (0.02)	0.49 (0.03)	<.001
	FA 2	Inferior fronto-occipital fasciculus L Inferior longitudinal fasciculus L & R Uncinate fasciculus L Superior longitudinal fasciculus (temporal part) L	684	−50	−25	1	0.43 (0.03)	0.39 (0.04)	0.043
CM > CF	FA 3	Anterior thalamic radiation L & R Corticospinal tract L & R	150	6	−33	−25	0.63 (0.04)	0.58 (0.04)	0.040
CF > AF	FA 4	Anterior thalamic radiation L & R Corticospinal tract L & R Cingulum (cingulate gyrus) L & R Cingulum (hippocampus) L & R Forceps major Forceps minor Inferior fronto-occipital fasciculus L & R Inferior longitudinal fasciculus L & R Superior longitudinal fasciculus L & R Uncinate fasciculus L & R Superior longitudinal fasciculus (temporal part) L & R	18,836	−15	36	3	0.54 (0.02)	0.51(0.02)	0.004
	FA 5	Anterior thalamic tract L Corticospinal tract L Forceps minor Inferior longitudinal fasciculus L Superior longitudinal fasciculus L	702	−11	−4	−3	0.70 (0.02)	0.68 (0.03)	0.045
	FA 6	Inferior fronto-occipital fasciculus L Inferior longitudinal fasciculus L Superior longitudinal fasciculus L Superior longitudinal fasciculus (temporal part) L	81	−47	−44	−3	0.56 (0.06)	0.52 (0.06)	0.049
CM > AM	FA 7	Anterior thalamic radiation L & R Corticospinal tract L & R Cingulum (cingulate gyrus) L & R Cingulum (hippocampus) L Forceps major Forceps minor Inferior fronto-occipital fasciculus L & R	18,386	19	35	11	0.56 (0.02)	0.52 (0.04)	0.003

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Comparison (Group 1 > Group 2)	Assigned Cluster Number	White Matter Tracts	Voxels	X (mm)	Y (mm)	Z (mm)	Group 1 *M* (*SD*)	Group 2 *M* (*SD*)	*p*
		Inferior longitudinal fasciculus L & R Superior longitudinal fasciculus L & R Uncinate fasciculus L & R Superior longitudinal fasciculus (temporal part) L & R							
	FA 8	Anterior thalamic radiation R Cingulum (hippocampus) R Forceps major Inferior fronto-occipital fasciculus R Inferior longitudinal fasciculus R Superior longitudinal fasciculus R	253	26	−53	18	0.62 (0.04)	0.58 (0.05)	0.042
	FA 9	Anterior thalamic radiation R Forceps major Inferior fronto-occipital fasciculus R Inferior longitudinal fasciculus R	105	23	−82	2	0.54 (0.05)	0.47 (0.06)	0.042

Coordinates reported are location of maximum intensity (peak) within the cluster. Cluster numbers are assigned for ease of reference in correlational analysis (Table S1). All comparisons were run bidirectionally (e.g. AUD > CON and CON >AUD) between the following groups: AUD vs. CON, M vs. F, CM vs. CF, AM vs. AF, CF vs. AF, CM vs. AM. Contrasts with significant differences are displayed above.

FA = fractional anisotropy, AUD = individuals with alcohol use disorder, CON = healthy controls, AF = females with AUD, AM = males with AUD, CF = female controls, CM = male controls

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