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White matter integrity in alcohol dependence and remission using tract-based spatial statistics

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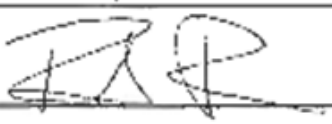

WHITE MATTER INTEGRITY IN ALCOHOL DEPENDENCE AND REMISSION USING TRACT-BASED SPATIAL STATISTICS

BY

MOLLIE MONNIG

B.A. ENGLISH

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science

Psychology

The University of New Mexico
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B.A., English, Amherst College, 2004
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ABSTRACT

The effects of alcohol on white matter integrity were investigated in a sample of 12 individuals with current alcohol dependence or abuse (AUD-C), 9 individuals with alcohol dependence in remission (AUD-R) for at least one year, and 16 healthy control (HC) participants matched to alcohol groups on age, sex, and smoking status. Magnetic resonance imaging (MRI) and neuropsychological data were collected. Diffusion tensor imaging (DTI) data was analyzed using tract-based spatial statistics (TBSS). Using a standard white matter atlas, fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were quantified for three corpus callosum ROIs and eight bilateral ROIs. Repeated measures ANOVAs revealed significant group differences for MD, AD, and RD and a trend for group differences in FA in the bilateral ROIs. Consequently, diffusivity measures for each ROI were tested for group differences using univariate ANOVAs followed by pairwise Tukey tests. Diffusivity was lower in AUD-C compared to HC in anterior limb of internal capsule, anterior corona radiata, cingulate gyrus, and external capsule. AUD-C also had lower diffusivity than AUD-R in

cingulum-hippocampal connections and uncinate fasciculus. Furthermore, correlations between frontal and limbic white matter and cognitive measures of executive function, attention, and processing speed were identified. Hypotheses of abnormal diffusion in frontal and limbic ROIs were confirmed, yet the direction of differences was opposite that of previous findings. These results draw attention to the critical issue of duration of abstinence and its possible role in contributing to the different pattern of white matter integrity observed across studies.

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Chapter 1: Introduction

Characterization of white matter tracts in a host of neurological and psychiatric disorders has recently received much attention due to the emergence of diffusion tensor imaging (DTI), an application of magnetic resonance imaging (MRI). This study investigated white matter integrity and its behavioral and neuropsychological correlates in groups of individuals with current alcohol use disorders (AUD) and with alcohol dependence in remission, compared to a healthy control group. A primary goal was to address questions of whether white matter integrity is compromised in relatively young alcohol dependent individuals and, if so, whether recovery is evident with sustained abstinence. Associations of white matter integrity with patterns of neuropsychological scores were also of principal interest.

Alcohol abuse and dependence are highly prevalent disorders in the US, affecting 4.65% and 3.81% of the population, respectively (Grant, Dawson et al., 2004). Because the effects of long-term alcohol abuse on the brain may have implications for treatment and prevention, characterizing those effects is of interest in alcohol research. Until recently, inquiry into brain changes associated with AUD relied upon postmortem examination of alcoholic individuals. However, developments in MRI technology now allow for *in vivo* quantification of the effects of alcohol on the brain.

Converging lines of evidence from MRI and neuropathological studies suggest that alcohol in excess exerts a neurotoxic effect with widespread ramifications. A large-scale, population-based MR study of alcohol consumption in participants approximately 60 years of age evinced a clear pattern of greater consumption being associated with

smaller cerebral volumes (C. A. Paul et al., 2008). The "high" drinking group, defined rather conservatively as those consuming 14 or more drinks per week, had significantly smaller volumes than former, light, and moderate drinkers and abstainers (C. A. Paul et al., 2008). A similar study reported a positive, dose-dependent correlation of sulcal and ventricular volumes with alcohol intake (Ding et al., 2004).

Reviews of neuropathological evidence have also indicated that alcohol dependence is associated with brain shrinkage, due primarily to loss of white matter and commensurate with level of alcohol intake (Harper & Kril, 1990). While alcoholics with Wernicke-Korsakoff syndrome, a disease caused by thiamine deficiency and associated with chronic alcoholism and malnourishment, manifest the most severe brain changes, non-Korsakoff alcoholics nevertheless show consistent deficits. For example, non-Korsakoff alcoholics as a group demonstrated significant reductions in brain weight and cerebral white matter upon postmortem examination, with 94% of the overall weight and 97% of the white matter volume of healthy individuals (Harper, Dixon, Sheedy, & Garrick, 2003).

Moreover, alcohol appears to differentially affect brain regions, with the frontal lobes, limbic system, and cerebellum most severely affected (Harper, 1998; Harper et al., 2003; Oscar-Berman & Marinkovic, 2007). Consequently, attention has turned to the characterization of white matter networks in alcohol dependence. In this pursuit, diffusion tensor imaging (DTI), an MRI method of ascertaining the microstructural integrity of white matter, has proven useful.

In DTI, a diffusion tensor which characterizes diffusion in three dimensions is measured. Commonly reported measures calculated from the diffusion tensor are mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA). AD is diffusivity in the direction of largest eigenvector of the diffusion tensor and is assumed to be aligned with the fiber bundle. RD is the mean value of the two smaller eigenvalues of the diffusion tensor perpendicular to the fiber bundle. MD is the mean value of all the eigenvalues. FA ranges from 0-1, where 0 indicates isotropic diffusion, or equal water diffusion in all directions, and 1 corresponds to total anisotropic diffusion, or diffusion only in a particular orientation. In white matter tracts, diffusion is bounded by the directional alignment of axonal bundles such that greater white matter coherence is generally reflected by higher FA and lower MD (Assaf & Pasternak, 2008; Pfefferbaum & Sullivan, 2005). Currently, MD elevations reported in alcoholism are thought to represent increases in extracellular and/or intracellular fluid, possibly due to moderate but widespread compromise of cytoskeletal integrity (Pfefferbaum and Sullivan, 2005). Decreases in FA can be caused by an increase in RD, typically associated with demyelination, or by a decrease in AD, typically associated with axonal injury. Animal experiments partially validating these observations have been reported by Song and colleagues (Song et al., 2003; Song et al., 2002; Song et al., 2005). Although the quality of axonal myelination certainly affects the DTI signal, other processes such as intracellular and extracellular edema (i.e., excess accumulation of water), gliosis, and inflammation can produce similar results, and so the exact nature of the pathology cannot be determined by DTI alone at this point (Assaf & Pasternak, 2008).

DTI is now being applied in the investigation of white matter changes in chronic alcoholism. One advantage of DTI is its ability to detect white matter disruption before changes are apparent on structural MRI, making it more sensitive than traditional volumetric measures (Pfefferbaum & Sullivan, 2005; Pfefferbaum et al., 2000). A seminal study by Pfefferbaum and colleagues (2000) reported white matter abnormality in alcoholism as measured by DTI in the absence of macrostructural volume loss. This and subsequent studies often have focused on the corpus callosum, the white matter tract connecting the left and right hemispheres. Some MRI volumetric studies have found shrinkage in the corpus callosum of alcohol-dependent individuals (Hommer et al., 1996; Pfefferbaum, Lim, Desmond, & Sullivan, 1996); others have failed to find macrostructural differences between alcohol and control groups (Pfefferbaum & Sullivan, 2002). Because of the high density of white matter in the corpus callosum (see Figure 1), differences between normal and alcoholic individuals should be readily apparent in this tract if in fact white matter is selectively damaged by chronic alcohol dependence. Of course, white matter differences do not necessarily reflect damage due to chronic alcohol exposure, as differences may be premorbid (see below).

In their study of corpus callosum and centrum semiovale, Pfefferbaum et al. (2000) found that FA corrected for age was significantly reduced in the genu (i.e., anterior corpus callosum) and centrum semiovale, but not splenium (i.e., posterior corpus callosum) in a group of alcoholic men compared to controls. A similar study performed with alcohol dependent women revealed decreased overall FA compared to healthy women, although the centrum semiovale was the only specific region showing a

significant difference (Pfefferbaum & Sullivan, 2002). When the alcoholic men and women were compared separately to controls from both studies, the alcohol groups demonstrated lower FA in genu and centrum than controls and did not differ from each other on these measures.

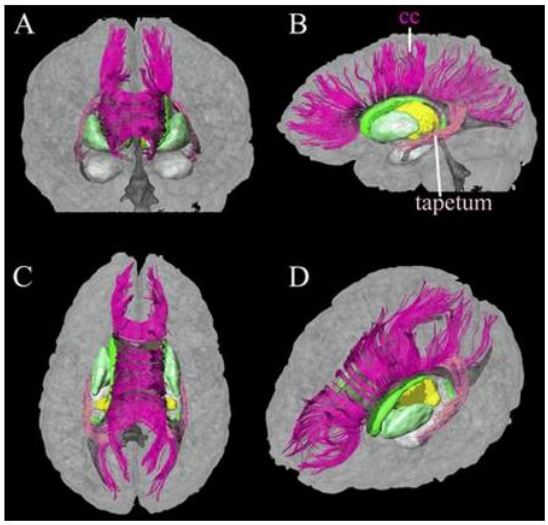

Figure 1. Depiction of callosal fibers from Wakana et al. (2004).

In a follow-up study, Pfefferbaum and Sullivan (2005) included MD analyses from the same subjects as above, citing the necessity of reporting both FA and MD in DTI studies. In this study, elevated MD was found in the genu and centrum semiovale, and MD in these regions was significantly, inversely correlated with FA. The authors made the case that FA deficits in the absence of increased MD primarily reflect disruption in the organization of white matter fibers, whereas an inverse relationship between FA and MD, that is, a strong link between reduced FA and increased MD, can be attributed to an excess in both intracellular and extracellular fluid. Based on diffusivity observations in animal models of stroke, Pfefferbaum and Sullivan (2005) speculated that the presence of

excess intracellular fluid is the predominant factor contributing to elevated diffusivity in alcohol dependence and that high correlations between FA and MD indicate a concomitant increase in extracellular fluid. Thus, on the basis of the inverse correlations between FA and MD identified in the study, Pfefferbaum and Sullivan (2005) concluded that "decreased brain white matter intravoxel coherence in alcoholism is attributable, at least in part, to the accumulation of interstitial or intracellular fluid or both fluid compartments, especially in the centrum, in excess of that occurring with advancing age" (p. 429).

Within the corpus callosum, it is not known why the anterior but not posterior portion apparently manifests alcohol-related damage. Interestingly, of eight regions examined in a cross-sectional study of normal aging, anterior corpus callosum exhibited the steepest linear decrease in FA with age, accompanied by a significant rise in MD (Hsu et al., 2008). In the same study, posterior corpus callosum showed a small positive relationship between MD and age but no significant age-related decrease in FA (Hsu et al., 2008). These observations would seem to suggest that, for reasons that are not yet clear, the anterior region of the corpus callosum may be differentially sensitive to the deteriorating effects of factors such as age and alcohol toxicity, as well as the interaction of age and alcoholism, as described below (Pfefferbaum, Adalsteinsson, & Sullivan, 2006a).

Although the corpus callosum is a natural target for investigation of white matter integrity, DTI tractography has enabled investigation of a wider array of white matter tracts. Harris et al. (2008) defined orbitofrontal cortex, cingulum bundle, and superior

longitudinal fasciculus (SLF) white matter as regions of interest (ROIs) based on a theorized "reward deficiency syndrome" in alcohol dependence, arising from damage to frontolimbic connections and specific to the right hemisphere. Reduced FA in the alcohol group relative to healthy controls was identified in these tracts, and deficits were in fact confined to the right hemisphere (Harris et al., 2008). However, selective damage to the right hemisphere in alcohol dependence has been disputed by other groups (Kwon, Rourke, & Grant, 1997), and it seems possible that the method used in this study of mirroring manually-defined right hemisphere ROIs to the left hemisphere could account for the lack of left hemisphere findings. The same study did not replicate findings of reduced FA in genu and centrum semiovale as first reported by Pfefferbaum et al. (2000). Harris et al. (2008) noted that ROIs were "relatively broadly defined" in that previous study, and so methodological differences might be responsible for the discrepancy (p. 7).

Recently, Pfefferbaum et al. (2009) utilized quantitative fiber tracking to investigate alcohol-related damage in 11 association fiber bundles in alcohol dependent individuals who had been abstinent an average of three months for men and six months for women. The authors found decreased FA in frontal forceps and superior cingulate and elevated diffusivity in these tracts as well as fornix, internal and external capsule, and superior longitudinal fasciculus (Pfefferbaum et al., 2009). In a study of alcohol dependence with a shorter and less variable time since last drink (6 ± 3 days), Yeh et al. (2009) identified clusters of decreased FA and/or increased diffusivity in a number of frontal, temporal, and limbic tracts associated with learning and memory, reward sensitivity, and inhibitory control. Frontal, limbic, and temporal areas with decreased FA

also tended to show significant increases in radial diffusivity, which the authors interpreted as evidence of demyelination (Yeh et al., 2009). On the other hand, increased MD in subcortical frontal white matter was paired with significantly increased axial diffusivity, which in these non-crossing fiber tracts may be suggestive of repair processes such as remyelination (Yeh et al., 2009).

Relationship of white matter damage to drinking history

As noted above, neuropathological studies have yielded evidence suggestive of ethanol-induced brain damage. In addition, a multitude of studies have demonstrated some normalization of brain structure and function with sustained abstinence, providing indirect support for the hypothesis that alcohol exerts a neurotoxic effect. However, establishing a direct relationship between alcohol consumption variables and white matter microstructural integrity as measured by DTI has proven less than straightforward.

Longitudinal studies are invaluable in characterizing the brain changes that accompany sustained abstinence. One study measuring brain volumes of alcoholics at one and six weeks of abstinence found an increase in brain volume and decrease in CSF in that relatively short period (Mann et al., 2005). Further, recently abstinent alcoholics who maintained abstinence over a period of several months showed reduced volume of the third ventricle and higher white matter volumes relative to those who resumed drinking (Pfefferbaum et al., 1995). Another comparison of relapsing and abstaining alcoholics via deformation-based morphometry identified many clusters spread throughout the brain, including areas in the anterior corpus callosum, temporal lobes, thalamus, and

cerebellum, in which abstaining alcoholics showed significantly greater tissue recovery, relative to baseline, than relapsing alcoholics (Cardenas, Studholme, Gazdzinski, Durazzo, & Meyerhoff, 2007). Overall, the rate of change in these clusters for relapsing alcoholics was -3.1 ± 2.8% over the several months between scans, compared to 7.7 ± 6.7% for abstinent alcoholics; as expected, light-drinking controls showed no change between scans (Cardenas et al., 2007).

A cross-sectional study comparing white and gray matter volumes in active heavy drinkers and recovering alcoholics with similar drinking and family histories found significantly greater white matter volume in the frontal lobes and a lower incidence of white matter lesions in the latter group (O'Neill, Cardenas, & Meyerhoff, 2001). Moreover, in the recovering alcoholics, who had been abstinent for approximately two years on average, a significant positive relationship was found between frontal white matter volume and duration of abstinence (O'Neill et al., 2001). On the other hand, recovering alcoholics demonstrated lower anterior cingulate gray matter volume and white matter volume in a remainder area compared to the actively drinking group (O'Neill et al., 2001). Another study noted no relationship between lifetime consumption and white matter volume or area of genu, splenium, or total corpus callosum; only time since last drink showed a significant, positive association with cortical white matter volume (Pfefferbaum, Rosenbloom, Serventi, & Sullivan, 2002).

In DTI studies, Pfefferbaum et al. (2000) noted that time since last drink was positively correlated with genu FA in their sample of alcoholic men, but no relationships were found between DTI measures and duration of alcoholism or lifetime consumption.

In a similar study on alcoholic women, genu FA and area were inversely correlated with lifetime consumption (Pfefferbaum & Sullivan, 2002). Importantly, when both variables were entered as simultaneous predictors in a regression to make allowance for the commonly found correlation between age and consumption, only consumption was a significant predictor (Pfefferbaum & Sullivan, 2002). Despite these promising leads, a consistent pattern of associations of drinking history with DTI measures has yet to emerge.

Of course, one cannot determine with certainty that alcohol consumption plays a causal role in white matter deficits, as any observed differences in the white matter of normal individuals versus alcohol dependent individuals may have existed prior to onset of problem drinking. In one study of alcohol dependent adults, subjects with positive family history, a risk factor for development of alcohol use disorders, had smaller intracranial volumes (Gilman, Bjork, & Hommer, 2007). Another study found no association between positive family history and reduced white or gray matter volumes in alcohol dependence (Cardenas, Studholme, Meyerhoff, Song, & Weiner, 2005). Since both of these studies looked only at alcohol dependent adults, they cannot address the question of premorbid differences.

Unfortunately, longitudinal studies investigating possible premorbid differences in at-risk individuals with differential rates of actual alcohol dependence have not been conducted. Looking at white matter characteristics in individuals with ADHD without comorbid alcohol use disorders may have some bearing on the issue of premorbid differences, as children with ADHD have a higher risk of developing alcohol use

disorders than the general population (Sullivan & Rudnik-Levin, 2001). In fact, white matter differences have been found in ADHD without comorbid alcohol problems that overlap to a certain extent with findings in alcohol dependence. Children with ADHD who were not taking medication showed significantly decreased total white matter volume compared to controls at approximately 10 years of age; volume deficits were not localized to a particular region but were present throughout the brain (Castellanos et al., 2002). A DTI study of children with ADHD found decreased FA in right frontal and striatal tracts and in left cerebellum (Ashtari et al., 2005). In adults with childhood ADHD, overall white matter actually showed a trend toward greater volume relative to controls, while overall gray matter, prefrontal, and anterior cingulate cortex volumes were smaller (Seidman et al., 2006). In contrast, DTI studies examining tracts underlying attentional networks nevertheless demonstrated reduced FA in the cingulum bundle and frontal portion of the SLF in adults with childhood ADHD (Makris et al., 2008).

Thus, it is possible that a shared, general risk factor for alcohol dependence includes reduced white matter integrity of certain tracts, even if evidence for overall white matter deficits in adults is ambiguous. Although beyond the scope of the current study, longitudinal investigations of children with and without high risk for alcohol dependence would be highly illuminating as to the nature of premorbid differences.

Neuropsychological impairment

Even in uncomplicated alcoholism, neuropsychological deficits can be related to the state of white matter microstructure. Frontal cortex, believed to be one of the most

seriously compromised brain region in AUD (Crews & Boettiger, 2009; Oscar-Berman & Marinkovic, 2007), is essential to normal attention and executive function (Fuster, 2002; Norman & Shallice, 1986). In addition, the vulnerability of thalamus, the brain's sensory relay area, and cerebellum, critical to both simple and complex motor function, would imply that more basic abilities might also show impairment.

Pfefferbaum et al. (2000) examined associations of white matter integrity with indices of attention and working memory. In alcoholic men but not healthy men, positive correlations were found between intervoxel coherence (that is, voxel-to-voxel directional coherence) in the genu and attention scores and between splenium FA and working memory scores. This same association was not found in a subsequent study on alcoholic women, however (Pfefferbaum & Sullivan, 2002). Pfefferbaum et al. (2007) found correlations between FA or MD and measures of motor ability in HIV-positive alcoholics, but not in those without comorbid HIV.

Few alcohol studies have been conducted that included neuropsychological testing in addition to DTI measures of tracts other than corpus callosum. Harris et al. (2008) found a significant, positive correlation between working memory and FA in left and right SLF in controls, which was apparently disrupted in alcoholics. The schizophrenia literature is farther along in linking white matter integrity as measured by DTI to neuropsychological function. Although the findings of DTI studies on schizophrenia samples cannot be applied directly to alcoholism, as one would expect different pathophysiology in each disorder, these studies can provide clues as to functional consequences of white matter damage. For example, lower cingulum FA has been linked

to worse executive functioning, as measured by nonperseverative errors on the Wisconsin Card Sorting Test (WCST); to poorer attention, as measured by the Attention Network Test; and to lower general intelligence, reflected in the four major indices of the Weschler Adult Intelligence Scale-III (Nestor et al., 2004; Nestor et al., 2008; Nestor, Kubicki, Spencer et al., 2007). In addition, lower uncinate fasciculus FA was found to be associated with poorer episodic memory scores on the Wechsler Memory Scale-Revised (WMS-R; Wechsler, 1987) in schizophrenia (Nestor et al., 2004; Nestor et al., 2008). Finally, FA of the fornix showed a positive relationship with performance on certain aspects of memory tests, specifically category fluency and semantic organization (Nestor, Kubicki, Kuroki et al., 2007). These results uphold traditional conceptualizations of the brain areas and connections involved in the cognitive processes studied and, as such, suggest that these same structure-function relationships may pertain to the study of white matter integrity in alcoholism.

Interactions with sex, age, and smoking status

Investigations of white matter changes in alcohol dependence must take into consideration possible interactions with sex and aging. A number of studies have posited a "telescoping effect" in which women with alcohol dependence manifest alcohol-related brain damage of a severity comparable to or greater than that found in men despite later disease onset, shorter duration, and lesser consumption (Hommer et al., 1996; Mann et al., 2005).

Although alcoholic women recruited for study do generally have later onset and considerably lower levels of consumption than their male counterparts, it is not firmly established that they suffer a similar extent of damage. A study comparing recently abstinent alcoholics to healthy controls within the sexes revealed volume deficits in cortical gray and white matter accompanied by sulcal and ventricular enlargement in the alcoholic men (Pfefferbaum, Rosenbloom, Deshmukh, & Sullivan, 2001). Strikingly, none of these brain differences were identified in the alcoholic women (Pfefferbaum et al., 2001). Additional analyses were performed on subsets of male and female alcohol groups matched on alcohol use history, that is, on alcoholic men with lighter consumption and later onset respective to the total alcoholic male sample and alcoholic women with heavier consumption and earlier onset respective to the total alcoholic female sample (Pfefferbaum et al., 2001). Absence of deficits persisted even in the heavy-drinking subgroup of women relative to healthy women, but the lighter-drinking group of men nevertheless had larger sulcal and ventricular volumes than healthy men (Pfefferbaum et al., 2001). Further, Pfefferbaum and Sullivan (2002) observed decreased splenium FA in alcoholic men compared to controls, which was not found when comparing alcoholic women to the same control group.

A study of supratentorial white matter of the left hemisphere, right hemisphere, and midline found significantly lower FA in alcoholic men compared to healthy men in 80%, 86%, and 71% of slices, respectively (Pfefferbaum, Adalsteinsson, & Sullivan, 2006b). The same analysis comparing alcoholic and healthy women yielded reduced FA in 39%, 13%, and 19% of slices, respectively (Pfefferbaum et al., 2006b). As these results

suggest, alcoholic men possessed a far greater proportion of slices with FA deficits than did alcoholic women (Pfefferbaum et al., 2006b). However, the authors' methodology allowed only for within-gender comparison of alcoholic dependent individuals to controls, and so it is not known whether men and women in the control groups exhibited differences in white matter integrity that might have influenced results. Studies of normal samples have identified higher FA in males than females in several clusters in frontal, temporal, and cingulate white matter (e.g., Hsu et al., 2008).

A more readily interpretable interaction is that of alcoholism and age. In one study, alcoholic men and women showed both FA and MD abnormalities in the splenium, genu, and body of the corpus callosum relative to healthy controls (Pfefferbaum et al., 2006a). When combined into one group, the alcoholic men and women demonstrated a negative correlation of FA and positive correlation of MD with age in the callosal body, as well as negative correlations between age and area of genu and splenium (Pfefferbaum et al., 2006a). These analyses utilized age-corrected scores for the alcohol group based on measures from the age-matched control group. Thus, the authors concluded that the changes in the alcohol group described above occurred in excess of those found in normal aging (Pfefferbaum et al., 2006a).

Use of an age-matched control group is essential in comparisons of alcoholic and nonalcoholic groups, as non-linear relationships have been identified in DTI measures in corpus callosum. In particular, evidence suggests that MD in the genu is best modeled by a quadratic function, wherein diffusivity continues to decrease in early adulthood until approximately age 35-40, at which point it begins to increase nonlinearly with advancing

age (Hasan et al., 2008; Pfefferbaum, Rosenbloom, Adalsteinsson, & Sullivan, 2007). In normal individuals, anterior corpus callosum showed the steepest negative slope for FA with increasing age out of a number of ROIs relevant to the study of white matter and aging (Hsu et al., 2008). As one might expect given the oftentimes inverse association of FA and MD, anterior corpus callosum also showed a significant positive correlation for MD and age. However, the relationship between MD and age described by Hsu et al. (2008) was linear, as opposed to the quadratic model stipulated in other studies, perhaps because the age range did not include younger adults under age thirty. As noted above, FA in posterior corpus callosum was not related to age, indicating that relationships between age and DTI measures vary by callosal segment (Hsu et al., 2008).

One additional, important consideration for investigation of group differences in white matter integrity is the potential confound of smoking status. Nicotine dependence occurs in 12.8% of the general population compared to 45.5% of alcohol-dependent individuals (Grant, Hasin, Chou, Stinson, & Dawson, 2004). Due to this increased prevalence of nicotine dependence in alcoholics, groups in alcohol studies often differ significantly on past and current smoking status.

The somewhat counterintuitive effect of smoking on white matter and its problematic nature in alcohol studies is now being recognized. Although smoking has been shown to reduce gray matter volumes of prefrontal and anterior cingulate cortex (Brody et al., 2004), it may actually increase white matter volumes in some brain areas, according to one study that divided alcoholic and light drinking groups into smoking and nonsmoking groups (Gazdzinski et al., 2005b). Alcoholics compared to controls had

reduced white matter in frontal and parietal lobes. Within the alcohol group, smoking alcoholics had reduced parietal gray matter but increased temporal lobe white matter compared to nonsmoking alcoholics (Gazdzinski et al., 2005b). Smoking and alcohol main effects consisted of reduced parietal and temporal lobe gray matter, but no interaction emerged (Gazdzinski et al., 2005b). Collapsing across smoking status in a post hoc analysis, Gazdzinksi et al. (2005b) identified significantly larger white matter volumes in smokers in temporal and frontal lobes. A number of relationships between white matter and neuropsychological test scores were found in nonsmoking alcoholics, including significant correlations between white matter volume of the temporal lobe and visuospatial learning and memory scores. Working memory also correlated with frontal and temporal lobe white matter volumes in this group. Interestingly, these relationships were absent in smoking alcoholics, suggesting a dissociation of structure-function relationships in smokers (Gazdzinski et al., 2005b). In other words, increased volumes associated with smoking may not confer functional benefits in smokers.

A recent study of corpus callosum in smokers, some of whom were regular drinkers but none of whom were daily drinkers, quantified volumes with voxel-based morphometry (VBM) and microstructural integrity of the corpus callosum using DTI (R. H. Paul et al., 2008). Smokers had significantly higher FA than nonsmokers in total corpus callosum, with increases specific to body and showing a trend in splenium, yet differences in callosal volumes were not evident (R. H. Paul et al., 2008). Smokers with a lower level of nicotine dependence had the highest FA levels of any of the subjects. Genu FA was positively correlated with number of cigarettes per day. The authors rejected the

idea that these results were attributable to edema, proposing instead that the "neurogenic properties of nicotine" may lead to "increased volume and microstructural integrity" of white matter (R. H. Paul et al., 2008). Neuropsychological measures were not administered in this study, so it cannot be determined whether the observed elevations in FA possessed functional relevance. Also, because alcohol intake was not quantified, relationships between smoking and drinking status and white matter could not be evaluated in this study.

In a study that quantified gray matter volumes using VBM, the authors found several well-replicated areas of gray matter deficit in people with schizophrenia (Tregellas et al., 2007). Additionally, the authors compared smokers and nonsmokers within the schizophrenia group, noting that smokers had higher gray matter volumes in superior temporal gyrus and lateral prefrontal cortex (Tregellas et al., 2007). When smoking status was included in the main analyses, gray matter differences between schizophrenics and controls were magnified (Tregellas et al., 2007). Unfortunately, the study did not include a smoking control group, which would be useful given findings of reduced gray matter in smokers without comorbid psychological disorders. The authors stress that smoking status may present a serious confound in studies attempting to characterize volumetric differences between groups with differential rates of smoking, an observation that could conceivably apply to alcohol dependence as much as schizophrenia. Animal studies could help to elucidate the effects of nicotine on white matter controlling for these confounds, yet studies applying DTI to animal models of nicotine consumption are lacking at present.

Variation in methods

Three main methods of obtaining DTI measures are currently in widespread use and will be described briefly. First is the manual tractography approach, which is described in detail in Wakana et al. (2007). Using a computer program such as DtiStudio, a rater specifies a region of interest (ROI) by delineating a seed area on the diffusion-weighted MR image, guided by neuroanatomical landmarks. Then the rater defines a second ROI, also according to landmarks, and instructs the program to create a representation of the tract connecting the two ROIs. A threshold is applied such that only areas with FA greater than a given value (e.g., FA = .2) are included in the ROI. At this point statistics (i.e., mean FA, MD, AD, and RD) can be computed for the tract. Wakana et al. (2007) established protocols and reported intra- and inter-rater reproducibility for 11 white matter tracts.

A second method utilizes a white matter atlas and does not require manual selection of ROIs. In this approach all subjects' diffusion-weighted images are registered to a standard white matter atlas, such as the one developed by Johns Hopkins University. Tracts are defined by the concordance of the individual's image and the atlas, and statistics are again computed for the mean FA, MD, RD, and AD of each tract.

Each approach has its virtues as well as its drawbacks. In the manual method, each tract is placed by hand, minimizing errors that often occur when many subjects' brains are registered to a standard atlas. On the other hand, manual placement of tracts is necessarily subjective and prone to rater drift or error. In Wakana et al. (2007), acceptable

reproducibility was achieved following a standard protocol, but the authors cautioned that reproducibility may be lower in different labs depending on the procedures followed. Also, the authors pointed out that their study used only normal individuals, and applicability of their results to clinical populations is not known.

The method of automatic registration to a standard white matter atlas has the advantage of bypassing subjective ratings and reducing the considerable time commitment entailed by the manual approach. On the other hand, Smith et al. (2006) noted two potential problems with this method. The first is the difficulty of ensuring that registration to standard space results in the same voxel-by-voxel placement for each subject, given individual differences in brain structure. This situation may be compounded in studies of clinical populations, where it is not only possible but actually expected that some individuals' brains will deviate from a "standard" brain. A second stumbling block is the fact that the degree of spatial smoothing applied to the data can exert a large effect on the results, and no decision rule for the appropriate degree of smoothing exists at this time.

Smith et al. (2006) proposed a third method, tract-based spatial statistics (TBSS), as a solution to these issues in DTI analysis. TBSS does not require hand placement of ROIs, nor does it rely on absolutely correct coregistration of images or require spatial smoothing. In this method, individuals' FA images are aligned to a white matter template. Then the mean of all FA images is thinned perpendicular to the tract of interest to construct a skeletonized, as opposed to fully volumed, mean FA image. Subjects can be compared on a voxel-wise basis or on the basis of the individual's mean skeleton values

for predetermined ROIs (Smith et al., 2006). In the present study, TBSS was selected as the method of choice, and analysis was performed on mean skeleton values for predetermined ROIs selected from a standard white matter atlas.

Hypotheses

Hypothesis 1. The first hypothesis stipulated that alcohol groups would demonstrate reduced white matter integrity relative to controls. On the basis of previous findings, we focused our investigation on white matter of corpus callosum, frontal and limbic fiber bundles (cingulum, fornix, uncinate fasciculus, internal and external capsule), and the superior longitudinal fasciculus.

Hypothesis 2. The second hypothesis stated that the alcohol groups would exhibit decrements on neuropsychological measures relative to controls. On the basis of previous studies, it was thought that the alcohol groups would show impairment on neuropsychological measures of attention, speeded processing, and executive functioning.

Hypothesis 3. It was further hypothesized that a gradient of impairment would exist such that participants in sustained remission from alcohol dependence would show lesser impairment on neuropsychological and neuroimaging measures than participants actively abusing alcohol.

Hypothesis 4. This hypothesis stated that relationships between white matter ROIs and neuropsychological functions would be found, e.g., that FA in the cingulum bundle would positively predict performance on measures of executive functioning.

Chapter 2: Methods

Participants

This study utilized a cross-sectional, three-group design with no follow-up phase. The Healthy Control (HC) group was a healthy normal comparison group with no history of alcohol abuse or dependence, recruited from the Albuquerque community. The current AUD group (AUD-C) comprised individuals with alcohol abuse or dependence active in the past month. The AUD in remission group (AUD-R) contained subjects with alcohol dependence in sustained full remission of at least one year so as to better understand the long term effects of alcohol dependence and abstinence. The alcohol groups were recruited through the Center on Alcoholism, Substance Abuse, and Addictions in Albuquerque, NM.

General inclusion criteria were as follows: 1) willingness to participate in all study components; 2) ability to provide informed consent; 3) ability to read, speak, and understand English at the 6th grade level; 4) ability to provide at least one contact person to assist with collateral interviews; 5) between the ages of 21 and 45; 6) at least 48 hours after last drink. General exclusion criteria were as follows: 1) history of neurological disorder or disease; 2) history of traumatic brain injury with loss of consciousness for more than 5 minutes; 3) medical illness severe enough to compromise participation in the study; 4) mental retardation, dementia, or other cognitive impairment of sufficient severity to render the individual incapable of informed consent; 5) active suicidality (i.e., any clinically significant suicide attempts in the past 3 months or any current suicidal

intent or definite plan); 6) current homicidal ideation aimed at a specific person or persons; 7) a first-degree relative with schizophrenia or other psychotic disorder.

All diagnoses were made using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID; First, Spitzer, Miriam, & Williams, 2002). Participants in the AUD-C group met criteria for alcohol dependence (n =11) or abuse (n = 1) active within the past 1 month (i.e., not in early or sustained full remission), and had engaged in two or more days of heavy drinking, defined as 5 or more drinks per occasion for a man or 4 or more drinks per occasion for a woman, within the 30 days prior to screening. Participants completed the Form-90 (Miller & Del Boca, 1994; Tonigan, Miller, & Brown, 1997), which captures the individual's drinking behavior during the 90 days preceding his or her most recent drink, in order to provide an index of drinking severity. Time between intake and scanning varied, and participants were a community sample rather than inpatients engaged in alcohol treatment. Consequently, time elapsed between last drink and scanning was not known for most participants. Participants in the AUD-R group were required to have SCID diagnosis of alcohol dependence not active within the past 12 months (i.e., sustained full remission). AUD-C and AUD-R participants were not excluded if they reported abuse of non-alcohol substances or other Axis-I disorders, but they were required to provide a urine sample free of illicit drugs before beginning study procedures.

Comorbid diagnoses of non-alcohol substance abuse or dependence and other Axis-I disorders were common in AUD-C and AUD-R groups. Only current (that is, present within the past month) diagnoses for non-substance use disorders are presented.

In the AUD-C group, 4 met SCID criteria for mood disorders, 7 for anxiety disorders, and 1 for an eating disorder. In the AUD-R group, 2 met SCID criteria for mood disorders, and 3 met SCID criteria for anxiety disorders. In both groups, some participants met criteria for more than one anxiety disorder diagnosis.

Both current and retrospective diagnoses for non-alcohol substance abuse or dependence are reported. In the AUD-C group, diagnoses for non-alcohol substances were as follows: 5 cannabis abuse/dependence (4 history, 1 current); 2 stimulant dependence (history); 1 opioid abuse (history); 7 cocaine abuse/dependence (6 history, 1 current); 1 hallucinogen abuse (history); 1 other drug abuse (history). For the AUD-R group, non-alcohol substance use diagnoses were as follows: 1 sedative abuse (history); 4 cannabis abuse/dependence (history); 3 stimulant dependence (history); 2 opioid dependence (history); 5 cocaine abuse/dependence (history); 2 hallucinogen abuse (history). Note that the majority of non-alcohol substance use diagnoses in the AUD-C group and all in the AUD-R group were not current. AUD-R participants had been in sustained remission from alcohol dependence an average of 3 years (range = 1-6 years).

The majority of participants (n = 12) in the HC group were screened using the SCID and excluded if they reported current or past substance abuse or dependence or other Axis-I psychopathology. However, attempts to match groups on smoking status, which may affect white matter (Brody et al., 2004; Gazdzinski et al., 2005b; C. A. Paul et al., 2008), necessitated addition of control subjects (n = 4) whose data was collected under a different protocol. These subjects did not complete the SCID but were screened for alcohol and drug use disorders using the Alcohol Dependence Scale (ADS; Skinner &

Horn, 1984), the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993), and in-house alcohol and drug consumption questionnaires.

Neuropsychological Assessment

Executive functioning, attention, and speeded processing are among the neuropsychological domains consistently implicated in chronic alcohol dependence (Chen et al., 2007; Crews & Boettiger, 2009; Fein, Torres, Price, & Di Sclafani, 2006; Oscar-Berman & Marinkovic, 2007; Oscar-Berman, Kirkley, Gansler, & Couture, 2004). It was hypothesized that these domains would be associated with integrity of frontal and limbic ROIs. Executive function was assessed with the Wisconsin Card Sorting Test (WCST), attention with Conners' Continuous Performance Test (CPT; Conners, 1994), and speeded processing with the Trail Making Test, Parts A & B (Reitan, 1992). See Lezak (2004) for a comprehensive review of these neuropsychological tests.

The WCST is a widely used neuropsychological test of executive function in which the individual is told to match a given stimulus to one of four options. Criteria for matching the stimuli are not supplied, but the individual is told whether the match is right or wrong for each turn. After ten consecutive correct trails, the matching criterion changes, e.g., from color to number. This test highlights the individual's ability to make decisions and alter behavioral responses in light of changing feedback from the environment. Commonly used performance indices are total errors and perseverative errors. In the following analyses, a composite formed by averaging age- and education-adjusted t-scores for these two variables was used.

The CPT is a test of attention in which letters appear at varying intervals on a computer screen, and the individual is instructed to respond by pressing the spacebar as quickly as possible for every letter except X. Outcome measures from this test that strongly reflect attentional capacity include number of omissions, that is, failure to respond to targets; reaction time standard error, which assesses response speed consistency; and variability, which also assesses response speed consistency but factors in alteration in response speed across segments of quicker or slower stimulus presentation in relation to overall response speed standard error. A composite formed by averaging age-adjusted t-scores for these variables was used.

The Trails A & B tests are used to evaluate visuospatial processing speed. Trails A, the simpler of the two tests, presents the examinee with numbered circles arrayed across a page and asks the examinee to connect them in order as quickly as possible. Trails B is slightly more complex in that the stimuli include letters, and the individual is told to connect the circles by alternating number, letter, number, and so forth, again as quickly as possible. Thus, Trails A is a more straightforward test of visuospatial processing, whereas Trails B also incorporates an element of speeded set-shifting between two different types of stimuli.

Effects of age and education were controlled for. For the WCST and Trails, t-scores adjusted for both age and education are available, and these scores were used in analyses. For the CPT, only age-adjusted t-scores were available, so education was regressed onto the composite score and the residuals saved. Correlations of DTI measures with the CPT were performed using these residuals. Finally, because lower t-scores

indicate better performance in the original program, the CPT composite residual was reverse-scored for consistency with other measures, such that a higher number indicated better performance.

The rationale of using composites rather than individual scores is twofold. First, composites reduce the number of tests performed and consequently reduce the likelihood of Type I error. Further, a composite of two or more scores is a more reliable indicator of an examinee's ability than any single score. Thus, it was hoped that the use of composites would yield more stable, generalizable results. Groups were combined to investigate associations of neuropsychological performance with DTI measures due to small group sizes.

Both strategical thinking and sensitivity to environmental feedback, as assessed by the WCST, are known to rely heavily on frontal lobes (for review see Fuster, 2002). Converging evidence from imaging has shown reduced activity associated with impulsivity in the frontal lobes of individuals with AUD (Chen et al., 2007). In addition, error monitoring, critical to performance in the WCST, Trails, and CPT, is dependent on the function of the cingulate gyrus (e.g., Carter, 1998; O'Connell et al., 2007). ROIs connecting limbic and frontal areas, such as the anterior limb of the internal capsule, anterior corona radiata, and fornix (albeit indirectly), could plausibly subserve executive and attentional functions. As the connection between the hemispheres, the corpus callosum would be expected to play a role in tests requiring synthesis of a number of different functions, such as Trails A and B. Anterior corpus callosum, which allows for connection between corticocortical frontal connections, was also hypothesized to underlie

WCST performance. Thus, there was a priori reason for inclusion of ALIC, ACR, CGC, CHP, FX/ST, GCC, BCC, and SCC. However, the function of the EC is largely unknown, and SLF and UNC are believed to participate in memory, so inclusion of these ROIs was exploratory in nature.

MRI Procedures

All MRI scans were performed on a Siemens 3T Trio TIM scanner using the standard 12-channel phased array head coils provided with the system. Sagittal T1-weighted anatomical images were obtained with a multi-echo 3D MPRAGE sequence [TR/TE/TI = 2530/1.64, 3.5, 5.36, 7.22, 9.08/1200 ms, flip angle = 7°, field of view (FOV) = 256 x 256 mm, matrix = 256 x 256, 1 mm thick slice, 192 slices, GRAPPA acceleration factor = 2].

The DTI data was collected along the anterior commissure/posterior commissure line, with FOV = 256 x 256 mm, 128 x 128 matrix, slice thickness of 2 mm (isotropic 2 mm resolution), NEX = 1, TE = 84 ms and TR = 9000 ms. A multiple-channel radiofrequency (RF) coil was used, with GRAPPA (X2), 30 gradient directions, b = 800 s/mm^2, and b = 0 experiment repeated five times (Jones, 2004; Jones, Horsfield, & Simmons, 1999). The total imaging time was approximately 6 minutes. The sequence was repeated twice to improve signal-to-noise ratio for the majority of subjects. For the four subjects added to balance groups on smoking status, a single DTI sequence was performed.

Our DTI experiment consisted of 30 directions with b = 800 s/mm^2 and 5 repetitions with b = 0 s/mm^2. The b = 0 s/mm^2 measurements are interleaved, meaning that we began

with b = 0 s/mm^2 measurement, and repeated it after every six b = 800 0 s/mm^2 measurements. When the DTI experiment was repeated twice, the data was concatenated into one 4D data set and a concatenated table of corresponding b-value and gradient direction tables was also calculated. The gradient direction vectors corrected for image orientation were stored in the Siemens dicom files and extracted by the dicom2nii program (www.sph.sc.edu/comd/rorden/dicom.html). The DTI data processing consisted of the following steps:

DTI Quality Check. The DTI data quality was checked for a) signal dropout due to subject motion, producing striated artifacts on images; b) excessive background noise in the phase encoding direction, due to external RF leakage in the MRI scan room or to subject motion; c) signal dropout in the posterior regions of the brain due to scanner or head vibration; and d) large amounts of motion in the absence of signal dropout. If for a specific gradient direction any slice was found to have a problem, we decided to exclude the whole volume rather than specific slices. Our assumption was that head movement compromised the entire volume. After the automatic quality check, we manually inspected all datasets, checking for any additional problems. We confirmed the correctness of the automatic DTI quality check and excluded any further gradient directions with problems that were missed by the algorithm. The gradient directions with problems were removed and the gradient direction tables adjusted accordingly.

Due to concerns about the possibility for differential signal-to-noise ratios (SNR) across clinical and control groups, white matter SNR was calculated for each subject. White matter SNR was defined as an average of the white matter signal in genu and

splenium of the corpus callosum divided by the mean noise in the image calculated from the regions outside the brain. The regions outside the brain were taken in the read-out direction so as not be confounded by motion artifacts in the phase encoding direction. The signal in the corpus callosum was found by averaging over a manually selected cube with 6 mm sides. The SNR (mean ± SD) was 15.6 ± 2.4 for the control group, 13.1 ± 1.8 for the AUD-C group, and 15.8 ± 2.3 for the AUD-R group. Although the AUD-C group had lower SNR, it was close to the same order of magnitude.

A comparison was also made of the estimated motion for our three subject groups. We estimated subject motion by registering the DTI images to a b = 0 template. From the translation parameters δx, δy, and δz a mean displacement $\delta d = \sqrt{\delta x^2 + \delta y^2 + \delta z^2}$ was calculated for each gradient direction. During the course of the experiment, subjects typically settled to a mean position. A summary parameter of motion intensity was calculated as the standard deviation of δd. A mean and standard deviation of δd were then calculated. We dropped gradient directions with δd greater than 4 from the mean. The motion was similar for the three groups, except for three subjects with larger amounts of motion in the AUD-C group. After motion quality control, motion was reduced from uncorrected levels.

Motion and eddy current correction. After the above data pruning we had one 4D DTI volume and a table of corresponding b-values and gradient direction vectors. Next we found a good quality b = 0 s/mm^2 image template and registered all the images to this template. The first image was with b = 0 s/mm^2, but it can have artifacts and is not necessarily the best image for registrations of all the images. We took advantage of the

fact that we had interleaved b = 0 s/mm^2 measurements across the experiment by doing the registration in two steps. We first registered the b = 800 s/mm^2 to the nearest b = 0 s/mm^2 image with an affine 12 degrees of freedom (dof) and mutual information cost function and registered that nearest b = 0 s/mm^2 image to the template selected earlier with a rigid body 6-dof transformation with a correlation cost function. This choice is appropriate because the b = 0 s/mm^2 images have similar contrast and do not have distortions because of diffusion gradients. These two transformations are mathematically combined and applied once to the b = 800 s/mm^2 image. The rotation part of the transformation is then extracted and each gradient direction vector corrected for image rotation. All the image registration and transformations were done with the FLIRT [FMRIB's Linear Image Registration Tool) program [FMRIB Software Library (FSL); www.fmrib.ox.ac.uk/fsl], and the detection of outliers and data pruning was done with a custom program written in IDL (www.ittvis.com).

Calculation of diffusion tensor. The diffusion tensor, scalar diffusion parameters (MD, AD, RD, and FA) were calculated using dtifit (FSL).

Image registration for group analysis. The FA image was aligned to a FA template with a nonlinear registration algorithm, FNIRT (FMRIB's Nonlinear Image Registration Tool; FSL).

Region-of-interest analysis. Mori et al. (2008) have made an average white matter atlas based on the International Consortium for Brain Mapping template (Mazziotta et al., 1995), which consists of 50 regions and can be used for ROI analysis (Mori et al., 2008). Our hypotheses led to the selection from this atlas of three midline ROIs of the corpus

callosum and eight bilateral ROIs in frontal, limbic, and association fiber ROIs. The callosal ROIs were genu (GCC), body (BCC), and splenium (SCC). The eight bilateral ROIs included in the present investigation were as follows: anterior corona radiata (ACR); anterior limb of internal capsule (ALIC); cingulum, cingulate gyrus part (CCG); cingulum, hippocampal part (CHP); external capsule (EC); fornix/stria terminalis (FX/ST); superior longitudinal fasciculus (SLF); and uncinate fasciculus (UF).

Statistical Analyses

Hypothesis 1 of group differences in white matter integrity was tested using repeated measures ANOVA in which group membership was be entered as the between-subjects factor and FA, MD, AD, or RD of each ROI (averaged across left and right hemispheres) as the within-subjects factor.

Hypothesis 2 was tested using group membership as the between-subjects factor in ANOVAs of neuropsychological test scores. Findings of group differences were followed by pairwise Tukey tests to determine which groups differed significantly.

Hypothesis 3 was addressed by the t-tests described above to determine the nature of group differences following findings of a significant between-subjects factor.

Hypothesis 4 was tested by forming composite scores of standardized neuropsychological test scores purported to reflect attention, processing speed, and executive functioning, and then assessing the contribution of white matter ROIs thought to underlie those functions through

Chapter 3: Results

Hypothesis 1

Sixteen HC, twelve AUD-C, and ten AUD-R participants underwent study procedures. One AUD-R participant's data was excluded due to artifact, leaving nine AUD-R participants in all DTI analyses. Groups did not differ significantly on age, sex, or current smoking status. Participants were designated as smokers if they had smoked at least 50% of days during the 90-day period prior to screening. Groups did differ significantly in years of education, with the control group having attained a higher level of education than both alcohol groups. Consequently, education was used as a covariate in omnibus tests. See Table 1 for summary of demographic characteristics.

Table 1. Participant demographic characteristics (M ± SD)

	HC (n = 16)	AUD-C (n = 12)	AUD-R (n = 9)	Test statistic	*p*-value
Education	15.13 ± 1.71	12.33 ± 2.57	13.56 ± 2.60	$F(2,34) = 5.418$	.009
Age	32.56 ± 7.48	34.83 ± 7.37	36.44 ± 5.73	$F(2,34) = .930$	.404
Sex	9 m, 7 f	7 m, 5 f	7 m, 2 f	$\chi^2 = 1.246$	.536
Smoking status	10 ns, 6 s	6 ns, 6 s	3 ns, 6 s	$\chi^2 = 1.974$	.373

Note: HC = healthy control; AUD-C = current alcohol use disorder; AUD-R = alcohol use disorder in remission.

Omnibus tests. Separate omnibus MANOVAs were performed on FA, MD, AD, and RD for the bilateral ROIs averaged across hemisphere and for the callosal tracts.

Bilateral ROIs were averaged across left and right hemispheres following the absence of group by hemisphere interactions for any measure in preliminary analyses. Therefore, in each MANOVA, group was entered as the between-subjects factor, ROI as the within-subjects factor, and education as a covariate. Means and standard deviations for DTI measures of callosal and bilateral ROIs are presented in Appendix A.

MD of the bilateral ROIs showed a significant effect of group [$F(2,33) = 4.708$, $p = .016$]. Likewise, the effect of group was significant for AD [$F(2,33) = 3.331$, $p = .048$] and for RD [$F(2,33) = 4.203$, $p = .024$]. The MANOVA on FA of the eight bilateral ROIs showed a trend for group [$F(2,33) = 2.671$, $p = .084$]. MANOVAs of the eight bilateral ROIs did not result in significant group by ROI interactions for any DTI measure.

The effect of group on callosal AD demonstrated a trend toward significance [$F(2,33) = 2.571$, $p = .092$). Examination of mean AD across the three callosal ROIs indicated that the largest difference was between AUD-C and HC groups, with AUD-C tending to have lower AD in the corpus callosum than HC. MANOVAs on callosal ROIs did not yield a significant effect of group on FA, MD, or RD. MANOVAs of the three callosal ROIs indicated that group by ROI interactions were not significant for any DTI measure.

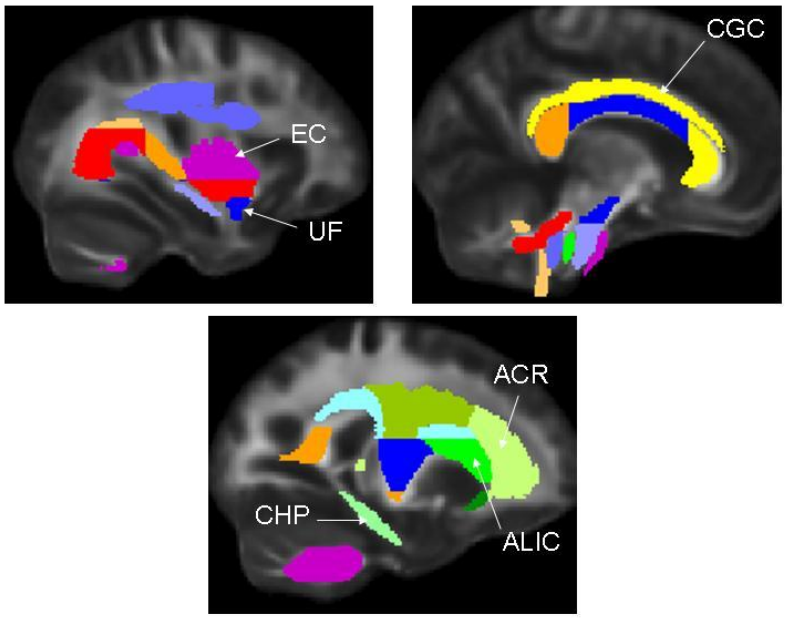

Figure 2. Illustration of white matter ROIs (labeled) with significant group differences. ACR = anterior corona radiata; ALIC = anterior limb of internal capsule; CGC = cingulum (cingulate gyrus) CHP = cingulum (hippocampal part); EC = external capsule; UF = uncinate fasciculus.

Post hoc tests. Pursuant to findings of significant group differences on MD, AD, and RD, univariate tests were performed on the eight bilateral ROIs. Following significant differences among groups for a particular ROI, pairwise contrasts were performed using Tukey's Honestly Significant Difference (HSD) test to correct for multiple comparisons. Compared to HC, the AUD-C group had significantly lower MD in ALIC and CGC and lower AD in ACR and EC. Relative to the AUD-R group, the AUD-C group showed significantly lower MD in CHP and lower RD in CHP and UNC. The HC and AUD-R groups differed from each other only on RD of the UNC, for which the AUD-R group had a significantly higher value than HC. Figure 2 illustrates ROIs with significant group differences. See Appendix B for a summary of ANOVA and contrast statistics and Figure 3 for graphical depiction of significant group differences.

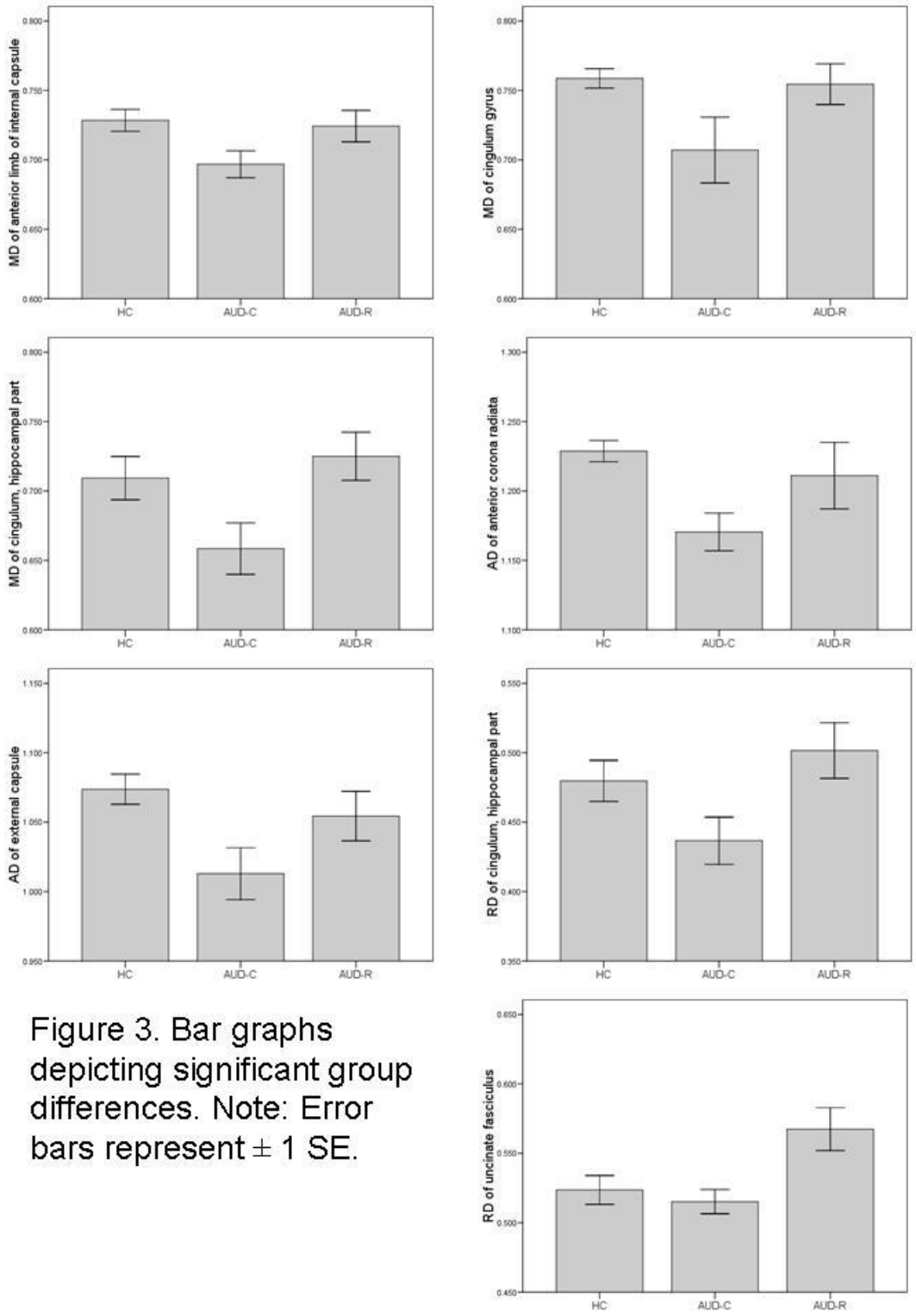

Figure 3. Bar graphs depicting significant group differences. Note: Error bars represent ± 1 SE.

Hypothesis 2

Neuropsychological data was available for 33 participants. Composition of the AUD-C and AUD-R groups remained the same, but the healthy control group was diminished by four participants. Demographics for the 12 HC participants with neuropsychological data were as follows: 30.08 ± 6.71 years of age, 12.33 ± 2.57 years of education, 6 female.

Table 2. Intercorrelations among variables used to form CPT and WCST composites

	CPT omissions t-score	CPT RT error t-score	CPT variability t-score	WCST total errors t-score	WCST perseverative errors t-score
CPT omissions t-score	—	r = .262, p = .141	r = .390, p = .025	—	—
CPT RT error t-score		—	r = .819, p < .001	—	—
CPT variability t-score			—	—	—
WCST total errors t-score				—	r = .915, p < .001
WCST perseverative errors t-score					—

Note: CPT = Conners' Continuous Performance Test; WCST = Wisconsin Card Sorting Test.

Intercorrelation between the variables that formed the WCST composite (total errors t-score and perseverative errors t-score) was strong and highly significant ($r = .915$, $p < .001$). For the CPT variables (omissions t-score, standard error of reaction time t-score, and variability t-score), intercorrelations ranged more widely. Standard error of reaction time and variability were highly correlated ($r = .819$, $p < .001$), and omission and variability were moderately correlated ($r = .390$, $p = .025$). On the other hand, omissions and standard error of reaction time showed a weak correlation that failed to meet significance ($r = .262$, $p = .141$). Nevertheless, composite makeup was not changed because this combination of variables was deemed to be of theoretical and clinical interest. Table 2 reports correlations of indices within each composite.

Table 3. Correlations among Trails A, Trails B, CPT, and WCST measures for all participants with neuropsychological data (n = 33)

	Trails A	Trails B	WCST	CPT
Trails A	—	r = .368, p = .035	r = .139, p = .439	r = .262, p = .141
Trails B		—	r = .364, p = .037	r = .334, p = .058
WCST			—	r = .290, p = .102
CPT				—

Note: Trails A = Trail Making Test Part A t-score; Trails B = Trail Making Test Part B t-score; WCST = Wisconsin Card Sorting Test composite; CPT = Conners' Continuous Performance Test composite.

Correlations among neuropsychological measures were relatively modest when present. Trails A & B were correlated with each other ($r = .368$, $p = .035$). Trails B was also correlated with WCST ($r = .364$, $p = .037$), which is suggestive of the higher-order

set-shifting abilities involved in Trails B. The correlation of Trails B and CPT showed a trend toward significance (r = .334, p = .058). See Table 3 for results.

Groups differed significantly from each other only on the CPT composite [F(2,30) = 4.931, p = .014]. Follow-up Tukey pairwise tests indicated that the HC group differed from the AUD-R group (p = .011), with the latter performing worse on the CPT. Other pairwise differences were not significant. See Table 4 for descriptive statistics and results of omnibus ANOVAs.

Table 4. Descriptive statistics and omnibus ANOVA results for neuropsychological variables

	HC	AUD-C	AUD-R	F-statistic	p-value
Trails A t-score	50.42 ± 11.12	53.67 ± 10.34	52.00 ± 8.38	.307	.738
Trails B t-score	55.67 ± 12.55	49.58 ± 9.40	47.56 ± 9.61	1.701	.200
CPT	.58 ± .85	-.11 ± .92	-.63 ± .88	4.931	.014
WCST	52.63 ± 11.05	46.21 ± 13.49	45.78 ± 10.99	1.161	.327

Note: Trails A = Trail Making Test Part A t-score; Trails B = Trail Making Test Part B t-score; WCST = Wisconsin Card Sorting Test composite; CPT = Conners' Continuous Performance Test composite. HC = healthy control; AUD-C = current alcohol use disorder; AUD-R = alcohol use disorder in remission.

Hypothesis 3

This hypothesis was tested in the post hoc tests following omnibus tests of Hypotheses 1 and 2.

Hypothesis 4

Bivariate correlations of FA, MD, AD, and RD of each white matter ROI with age- and education-adjusted neuropsychological scores are presented in Appendix C. For FA, positive correlations of moderate magnitude were identified between ACR and Trails B, ALIC and Trails A, ALIC and WCST, CHP and Trails A, GCC and WCST, and SLF and WCST. No correlation between ROI FA and CPT was significant.

For MD, moderate, negative correlations were found between ALIC and Trails A, CHP and Trails A, and FX/ST and Trails A. In addition, FX/ST MD was positively correlated with WCST. For AD, positive correlations emerged between ACR and CPT, EC and WCST, and GCC and CPT. For RD, negative correlations were found between ALIC and Trails A, BCC and WCST, CHP and Trails A, FX/ST and Trails A, and GCC and WCST.

Chapter 4: Discussion

Summary of findings

At least one diffusivity measure was lower in the currently drinking group compared to healthy controls in ALIC, ACR, CGC, and EC. Comparisons of current drinkers to those in remission yielded lower diffusivity in the current drinkers on CHP and UNC. Moreover, FA averaged across ROIs showed a trend for group differences, with the lowest mean FA found in the AUD-R group and the highest mean FA in the AUD-C group. Findings of significantly decreased diffusivity in individuals with current AUD compared to controls are contrary to previous studies (Harris et al., 2008; Pfefferbaum et al., 2006a, 2006b; Pfefferbaum et al., 2002; Pfefferbaum and Sullivan, 2005; Pfefferbaum et al., 2000; Yeh et al., 2009).

Even though the direction of the FA and MD differences for the AUD-C group was contrary to previous studies, the areas implicated by our analyses are consistent with reviews suggesting vulnerability of frontal and limbic regions to alcohol-related abnormality. Bidirectional frontothalamic fibers, cingulate gyrus, and external capsule manifested decreased diffusivity in current drinkers relative to healthy controls.

Neuropsychological assessment revealed group differences only on the attention composite, on which the AUD-R group performed significantly more poorly than the HC group. Across groups, significant correlations emerged between neuropsychological scores and DTI measures of white matter integrity in ALIC, ACR, BCC, FX/ST, CHP, EC, GCC, and SLF.

Although conclusions about the role of various ROIs in cognition remain weak due to small samples and correlational analyses, a few broad patterns emerged. Trails A and WCST showed numerous significant correlations with white matter ROIs, but findings for Trails B and CPT were sparse. This result suggests that, in this combined sample, simple visuospatial processing speed and executive function bear a stronger relationship to the ROIs examined than do attention or set-shifting. As predicted, the most numerous correlations were demonstrated with frontal, limbic, and callosal tracts, suggesting that variation in white matter integrity in these areas does contribute to one's ability to quickly process information and select appropriate responses to one's environment.

A further observation is that correlations with FA and AD were positive, whereas those with MD and RD were largely negative. FA has frequently been found to correlate positively with measures of cognition. Most studies linking diffusivity measures to cognition have reported negative relationships. However, these studies typically report only MD. One could speculate on the basis of current findings that diffusion parallel to the axon (i.e., AD) facilitates cognition and diffusion perpendicular to the axon (i.e., RD) hinders it.

Finally, significant relationships between neuropsychological performance and white matter integrity provide an important check on the nature of DTI group differences. Our findings regarding neuropsychological performance and white matter closely resemble those established in the literature. In other words, although higher FA and lower MD were found in the currently drinking group, in contradiction of previous studies, it

nevertheless remained the case that higher FA and lower MD were associated with better cognitive performance. That the direction of findings was not reversed for neuropsychological performance as it was for group differences in DTI measures would seem to confer greater credibility on the latter.

Relationship of current findings to previous studies

Perhaps the most critical distinction between this study and others, and a possible explanation of the discrepancy in results, is our formation of separate AUD groups based on duration of abstinence. The current study divided alcohol participants into two groups based on duration of sobriety, with participants in the AUD-C group having had their most recent drink within days or weeks of scanning. In contrast, participants in the AUD-R group met a minimum requirement of one year sustained remission, with length of abstinence in this sample ranging from one to six years. According to the definitions applied here, most previous studies have grouped together participants with both early and sustained remission to form a single experimental group (e.g., Harris et al., 2008; Pfefferbaum et al., 2002; Pfefferbaum and Sullivan, 2005; Pfefferbaum et al., 2000). The result has been that participants with recent or remote alcohol use disorders have been placed in the same group for DTI analyses regardless of length of sobriety, which has ranged in length on the order of years rather than weeks, indeed varying by as much as 28 years (e.g., Harris et al., 2008).

It seems reasonable to assume that the effect of problematic alcohol consumption on white matter integrity differs as a function of time elapsed since active drinking.

Therefore, including all participants with current or past AUD in a single group regardless of length of sobriety may not be the most effective means of identifying these effects. In fact, because currently drinking and recovering alcohol dependent individuals manifested DTI effects in opposite directions in our study, combining participants with short- and long-term abstinence into a single group may actually obscure the effects of alcohol on white matter.

On the other hand, a study by Yeh et al. (2009) applied TBSS to investigation of white matter abnormality in a sample of drinkers with a much smaller range of short-term (6 ± 3 days) abstinence. Strikingly, our analyses corroborated white matter abnormality in many of the same areas, such as corona radiata, external capsule, anterior limb of internal capsule (but not others, such as superior longitudinal fasciculus, fornix, or corpus callosum). As noted, however, the directions of FA and diffusivity differences in the present study are largely opposite to that of Yeh et al. (2009). It is worth noting that the drinking participants in the Yeh et al. study were somewhat older (47.0 ± 7.6 years old) and more heavily male (10 male, 1 female) than our sample. In addition, Yeh et al.'s (2009) heavy drinking group was selected from a larger sample on the basis of uniformly very high levels of alcohol consumption (374 ± 143 drinks/month, a level achieved only by the two heaviest drinkers in our sample). Finally, a limitation noted by Yeh et al. (2009) was that their heavy drinkers smoked at a significantly higher frequency than the light drinking sample to which they were compared (i.e., 10/11 heavy drinkers, compared to 2/10 light drinkers, were smokers).

The current study is the first to report widespread FA increases and diffusivity decreases throughout the brains of adults with current AUD. De Bellis et al. (2008) found increased FA and reduced MD in corpus callosum of adolescents with alcohol use disorders at 63.2 ± 88.2 days since their last drink. Although those authors attributed the findings to accelerated myelination in the alcohol group (De Bellis et al., 2008), aberrant patterns of diffusion brought about by the toxic effects of alcohol could conceivably account for elevated FA and reduced MD in currently drinking individuals.

Candidate mechanisms

The precise mechanisms by which these disturbances may take place are at present a matter of speculation. Increased FA and decreased radial diffusivity have been found in disorders such as hydrocephalus, where mechanical pressure caused by ventricular enlargement apparently resulted in white matter compression accompanied by disturbed diffusivity parameters (Assaf et al., 2006). Furthermore, Wilde et al. (2008) found increased FA and reduced apparent diffusion coefficient and RD in adolescents with mild traumatic brain injury in a time span of 1-6 days following injury. The authors attributed these findings to cytotoxic edema in response to trauma (Wilde et al., 2008). Similarly, both Bazarian et al. (2007) and Mayer et al. (2010) reported increased FA and decreased diffusivity in several regions in adult mild TBI, within 72 hours or 21 days of injury, respectively.

Such findings raise the possibility that increased FA and decreased diffusivity in the days or weeks following an individual's last drink might be a marker of alcohol-

induced edema. Of course, other studies on TBI have found decreased FA and elevated diffusivity (e.g., Kumar et al., 2009; Rutgers et al., 2008), making this interpretation tenuous. Further, decreased diffusivity in the present study was not confined to RD but was approximately equally distributed across both AD and RD, indicating that diffusion was restricted both parallel and perpendicular to the axonal bundle in our currently drinking sample.

Animal studies applying DTI measures to alcohol-induced changes are lacking at present. However, Song and colleagues have reported a series of studies attempting to use DTI to differentiate axonal degeneration versus demyelination. Song et al. (2002) observed that rats bred to have poor myelination but intact axons showed an increase in radial but not axial diffusivity. Using a rodent model of retinal ischemia, Song et al. (2003) showed that the initial axonal degeneration was accompanied by decrease in axial but not radial diffusivity and that subsequent demyelination produced an increase in radial but not axial diffusivity. Finally, by administering and then withdrawing a neurotoxin to rodents, Song et al. (2005) found that radial diffusivity increased with demyelination, decreased with remyelination, and was not correlated with axonal injury. Song and colleagues posited on the basis of these studies a double dissociation whereby increased radial diffusivity selectively reflects demyelination and decreased axial diffusivity selectively indicates axonal injury.

Applying these heuristics to our data, we might speculate that the decrease in axial diffusivity in AUD-C relative to controls in external capsule and anterior corona radiata reflects axonal injury in the absence of demyelination. Moreover, higher radial diffusivity

in AUD-R in superior longitudinal fasciculus compared to AUD-C and in uncinate fasciculus compared to AUD-C and HC might be interpreted as demyelination in the AUD-R group or as remyelination in the AUD-C group. The pattern observed in the neuropsychological data wherein higher axial diffusivity and lower radial diffusivity were individually correlated with better cognitive performance are in line with this speculation. It should be kept in mind, however, that the animal studies by Song and colleagues did not specifically investigate the effects of alcohol and that highly controlled animal models of axonal injury and demyelination are unlikely to capture the complex set of circumstances surrounding brain changes in humans.

In that many participants experienced periods of sobriety punctuated by heavy drinking in the months preceding MRI acquisition, our AUD-C group may most closely resemble the relapsing subsets of alcohol dependent samples in studies such as Pfefferbaum et al. (1995) and Gazdzinski et al. (2005a). As Gazdzinski et al. (2005a) noted, volumes in individuals exhibiting this drinking pattern are "constantly fluctuating, presumably putting considerable stress on the brain tissue and on mechanisms regulating brain volume" (p. 271). Rapid rebuilding of white matter tissue and/or compression from ventricular expansion may account for increased anisotropy and decreased diffusivity in the current drinkers. Longitudinal research on the nature and timecourse of diffusion disturbances in AUD, particularly with groups matched on smoking status, would be useful to address this discrepancy.

Limitations and future directions

As noted, this study is limited by the absence of precise data on time since last drink for the AUD-C group. A further limitation of this study is its cross-sectional nature, which precludes direct attribution of white matter abnormality to alcohol consumption. Furthermore, groups were disparate not only on drinking status but also on diagnoses of other substance use disorders and psychiatric illness. Another limitation of this study is its small sample size, which may have limited our power to detect more subtle differences between controls and alcohol groups in tracts where abnormality has previously been reported, such as genu of corpus callosum and superior longitudinal fasciculus.

In spite of these limitations, this study contributed to understanding of white matter damage in AUD by differentiating between current AUD versus AUD in sustained remission. Given that abnormality was identified in many of the same regions as previous studies, it may be that restricted diffusivity associated with ongoing heavy alcohol use represents a preliminary step to elevated diffusivity found in groups of individuals with both short- and long-term abstinence. Studies investigating both the longitudinal time course of white matter changes in AUD as well as premorbid abnormality in populations predisposed to AUD would be well suited to answering the questions raised by the present study.

Appendix A: Descriptive statistics (M ± SD) for white matter regions of interest

ROI	Group	FA	MD	AD	RD
ALIC	HC	.597 ± .026	.728 ± .038	1.285 ± .044	.450 ± .034
	AUD-C	.603 ± .026	.697 ± .033	1.242 ± .061	.425 ± .029
	AUD-R	.585 ± .017	.724 ± .034	1.266 ± .047	.454 ± .031
ACR	HC	.471 ± .016	.789 ±.023	1.229 ± .031	.570 ± .023
	AUD-C	.465 ±.027	.761 ± .034	1.170 ± .047	.556 ± .037
	AUD-R	.454 ± .018	.794 ± .050	1.211 ± .072	.585 ± .041
BCC	HC	.709 ± .031	.830 ±.038	1.671 ± .056	.410 ± .043
	AUD-C	.694 ± .061	.829 ± .059	1.634 ± .074	.425 ± .088
	AUD-R	.683 ± .032	.844 ± .055	1.645 ± .069	.443 ± .056
CGC	HC	.608 ± .023	.759 ± .028	1.357 ± .054	.460 ± .027
	AUD-C	.602 ± .038	.707 ± .082	1.253 ± .176	.434 ± .047
	AUD-R	.580 ± .033	.755 ± .044	1.314 ± .078	.475 ± .039
CHP	HC	.539 ± .045	.709 ± .062	1.169 ± .088	.480 ± .059
	AUD-C	.558 ± .056	.658 ± 0.064	1.103 ± .102	.437 ± .059
	AUD-R	.510 ± .059	.725 ± .052	1.172 ± .085	.502 ± .060
EC	HC	.446 ± .034	.711 ± .038	1.074 ± .043	.529 ± .039
	AUD-C	.441 ± .027	.676 ± .041	1.013 ± .065	.508 ± .035
	AUD-R	.427 ± .032	.711 ± .039	1.054 ± .053	.539 ± .038
FX/ST	HC	.573 ± .020	.772 ± .038	1.318 ± .062	.499 ± .033
	AUD-C	.560 ± .028	.769 ± .017	1.294 ± .045	.507 ± .026
	AUD-R	.559 ± .028	.775 ± .045	1.309 ± .064	.508 ± .043
GCC	HC	.759 ± .032	.747 ± .048	1.580 ± .085	.331 ± .046
	AUD-C	.744 ± .044	.726 ± .059	1.504 ± .102	.337 ± .058
	AUD-R	.735 ± .029	.752 ± .061	1.547 ± .089	.355 ± .051
SCC	HC	.793 ± .015	.740 ± .025	1.633 ± .054	.295 ± .020
	AUD-C	.787 ± .031	.728 ± .038	1.593 ± .067	.296 ± .041
	AUD-R	.767 ± .024	.761 ± .034	1.630 ± .039	.327 ± .037
SLF	HC	.508 ± .024	.758 ± .021	1.213 ± .021	.531 ± .030
	AUD-C	.502 ± .034	.748 ± .024	1.187 ± .036	.529 ± .035
	AUD-R	.494 ± .026	.770 ± .038	1.214 ± .046	.548 ± .038
UNC	HC	.509 ± .040	.754 ± .043	1.215 ± .071	.524 ± .041
	AUD-C	.513 ± .031	.745 ± .038	1.206 ± .076	.515 ± .030
	AUD-R	.473 ± .038	.79 ± .048	1.235 ± .079	.567 ± .046

Note: FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity. ACR = anterior corona radiata; ALIC = anterior limb of internal capsule; BCC = body of corpus callosum; CGC = cingulum (cingulate gyrus part); CHP = cingulum (hippocampal part); EC = external capsule; FX/ST = fornix/stria terminalis; GCC = genu of corpus callosum; SCC = splenium of corpus callosum; SLF = superior longitudinal fasciculus; UNC = uncinate fasciculus. HC = healthy control; AUD-C = current alcohol use disorders; AUD-R = alcohol use disorders in remission.

Appendix B. ANOVA and contrast statistics for tests of group differences on white matter ROIs

				p-value of Tukey-corrected pairwise contrasts			
DTI measure	ROI	F-stat	*p*-value	HC vs. AUD-C	HC vs. AUD-R	AUD-C vs. AUD-R	Direction of difference
MD	ACR†	3.128	.057				
	ALIC*	3.448	.043	.043	ns	ns	HC > AUD-C
	CGC*	3.438	.044	.047	ns	ns	HC > AUD-C
	CHP*	3.702	.035	ns	ns	.046	AUD-R > AUD-C
	EC†	3.151	.056				
	FX/ST	.079	ns				
	SLF	1.695	ns				
	UNC†	3.092	.058				
AD	ACR*	5.020	.012	.009	ns	ns	HC > AUD-C
	ALIC†	2.496	.097				
	CGC†	2.893	.069				
	CHP	2.167	ns				
	EC*	4.483	.019	.014	ns	ns	HC > AUD-C
	FX/ST	.610	ns				
	SLF†	2.659	.085				
	UNC	.413	ns				
RD	ACR	2.034	ns				
	ALIC†	2.956	.066				
	CGC†	3.254	.051				
	CHP*	3.376	.046	ns	ns	.047	AUD-R > AUD-C
	EC	2.023	ns				
	FX/ST	.339	ns				
	SLF	.934	ns				
	UNC*	5.070	.012	ns	.031	.014	AUD-R > HC; AUD-R > AUD-C

Note: MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity. ACR = anterior corona radiata; ALIC = anterior limb of internal capsule; BCC = body of corpus callosum; CGC = cingulum (cingulate gyrus part); CHP = cingulum (hippocampal part); EC = external capsule; FX/ST = fornix/stria terminalis; GCC = genu of corpus callosum; SCC = splenium of corpus callosum; SLF = superior longitudinal fasciculus; UNC = uncinate fasciculus. HC = healthy control; AUD-C = current alcohol use disorders; AUD-R = alcohol use disorders in remission.

Appendix C. Bivariate correlations of DTI measures with neuropsychological variables

DTI measure	ROI	Trails A	Trails B	WCST	CPT
FA	ACR	ns	r = .350, p = .046	ns	ns
	ALIC	r = .390, p = .025	ns	r = .404, p = .020	ns
	BCC	ns	ns	r = .406, p = .019	ns
	CGC	ns	ns	ns	ns
	CHP	r = .560, p = .001	ns	ns	ns
	EC	ns	ns	ns	ns
	FX/ST	ns	ns	ns	ns
	GCC	ns	ns	r = .434, p = .012	ns
	SCC	ns	ns	ns	ns
	SLF	ns	ns	r = .357, p = .042	ns
	UNC	ns	ns	ns	ns
MD	ACR	ns	ns	ns	ns
	ALIC	r = -.369, p = .035	ns	ns	ns
	BCC	ns	ns	ns	ns
	CGC	ns	ns	ns	ns
	CHP	r = -.371, p = .033	ns	ns	ns
	EC	ns	ns	r = .393, p = .024	ns
	FX/ST	r = -.389, p = .025	ns	ns	ns
	GCC	ns	ns	ns	ns
	SCC	ns	ns	ns	ns
	SLF	ns	ns	ns	ns
	UNC	ns	ns	ns	ns
AD	ACR	ns	ns	ns	r = .397, p = .022
	ALIC	ns	ns	ns	ns
	BCC	ns	ns	ns	ns
	CGC	ns	ns	ns	ns
	CHP	ns	ns	ns	ns
	EC	ns	ns	r = .398, p = .022	ns
	FX/ST	ns	ns	ns	ns
	GCC	ns	ns	ns	r = .421, p = .015
	SCC	ns	ns	ns	ns
	SLF	ns	ns	ns	ns
	UNC	ns	ns	ns	ns
RD	ACR	ns	ns	ns	ns
	ALIC	r = -.387, p = .026	ns	ns	ns
	BCC	ns	ns	r = -.405, p = .019	ns
	CGC	ns	ns	ns	ns
	CHP	r = -.507, p = .003	ns	ns	ns
	EC	ns	ns	ns	ns
	FX/ST	r = -.371, p = .034	ns	ns	ns
	GCC	ns	ns	r = -.403, p = .020	ns
	SCC	ns	ns	ns	ns
	SLF	ns	ns	ns	ns
	UNC	ns	ns	ns	ns

Note: FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity. Trails A = Trail Making Test Part A t-score; Trails B = Trail Making Test Part B t-score; WCST = Wisconsin Card Sorting Test composite; CPT = Conners' Continuous Performance Test composite. ACR = anterior corona radiata; ALIC = anterior limb of internal capsule; BCC = body of corpus callosum; CGC = cingulum (cingulate gyrus part); CHP = cingulum (hippocampal part); EC = external capsule; FX/ST =

fornix/stria terminalis; GCC = genu of corpus callosum; SCC = splenium of corpus callosum; SLF = superior longitudinal fasciculus; UNC = uncinate fasciculus.

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