An Initial Investigation of Disrupted Intracortical Myelin as a Novel Brain Marker of Alcohol Use Disorder

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Abstract

Although disruption of cortical gray matter and white matter tracts are well-established markers of alcohol use disorder (AUD), this is the first study to examine the specific role of intracortical myelin (ICM; i.e., highly myelinated gray matter in deeper cortical layers) in AUD. The current study used a 3T MRI sequence optimized for high intracortical contrast to examine patterns of ICM-related MRI signal in 30 individuals with AUD and 33 healthy social drinkers. Secondary aims included exploring continuous associations with alcohol problem severity and examining sex differences. Surface-based analytic techniques were used to quantify ICM-related MRI signal for *a priori* region of interest analyses (20 bilateral regions) and exploratory vertex-wise analyses (using Cohen's d). Although the distribution of ICM-related signal was generally comparable between groups, the AUD group exhibited significantly ($p < .05$) greater ICM-related MRI signal in precuneus, ventromedial prefrontal cortex, posterior cingulate, middle anterior cingulate, middle/posterior insula, dorsolateral prefrontal cortex, and posterior cingulate, among other regions (Cohen's $d = .50-.75$, indicating medium magnitude effects). Significant positive correlations between ICM signal and AUD severity were found in several frontal, parietal, cingulate, and temporal regions ($r_s .25-.34$). No sex differences in ICM were observed. These findings provide initial proof-of-concept for examining ICM in relation to AUD. Understanding the pathophysiological mechanisms of these associations (e.g., neuroinflammation) and the clinical relevance of ICM is warranted.

Introduction

Structural and functional disruptions of the cerebral cortex are well-established indicators of alcohol use disorder (AUD) [1,2]. People who meet criteria for AUD have been found to exhibit reduced cortical thickness [3,4], reduced white and grey matter volume [5], as well as reduced volume in the frontal cortex and other cortical and sub-cortical regions [3,6,7] relative to people without an AUD. Cortical thickness deficits are related to a number of clinical indicators in AUD, including associations with degree of alcohol use and misuse [4,8], poor AUD treatment outcomes [3], and impaired inhibitory control in people with AUD [9]. Although it has been theorized that the brain reaches peak development at roughly twenty-five years of age [10], some literature suggests that white matter may have a more prolonged trajectory [11,12]. Various studies have found that while grey matter may peak in one's mid-twenties, heavily-myelinated white matter on the other hand, may not fully develop until the third, fourth, or fifth decade of one's life [11,13]. As a result of this deferred development, white matter remains inherently sensitive and susceptible to damage from the use of addictive substances, environmental effects, and physical insults [14]. Reasons for the extended vulnerability in white matter include its low level of blood supply [11,12,14] and the extensive energy requirements of oligodendrocyte function [11,12,14]. Increased focus on studying associations between brain myelination and substance use disorders is an important and clinically-significant priority.

Although cortical deficits in AUD are generally well-established, the cerebral cortex is highly heterogeneous with respect to types of neuronal tissue [15]. In particular, there is growing interest in characterizing the role of myelin in the cortex. Myelin serves many functions, including increasing action potential transmission speed, improving neuronal synchrony, increasing brain connectivity, decreasing refractory time, and supporting cognitive functions [11]. Although primarily concentrated in neuronal tracts in the white matter, myelinated axons are also found in the cortex, with deeper layers (IV-VI) being more heavily myelinated than the superficial layers (I-III) [16]. These deeper, myelinated layers of the cortex represent what has

been termed *intracortical myelin* (ICM) [16]. Similar to myelin in white matter, ICM reaches peak development between the third to fifth decade of one's life [11,13] and thus remains vulnerable to toxins and damage [11,17]. Intracortical myelin is thought to play a central role in synchronizing and speeding of neuronal signals through the cortex in support of cognitive processing. For example, research has shown that decreased ICM in posterior cingulate cortex in healthy individuals is correlated with decreased error processing and cognitive control [18] and reduced ICM in insula and superior temporal gyrus is associated with increased performance variability [19].

Since the magnetic resonance imaging (MRI) signal is very sensitive to the presence of myelin, several MRI protocols have been developed to image ICM content *in vivo* [20,21]. In the protocol developed by Bock et al. [21], the T1-weighted MRI contrast was optimized for differentiating cortical regions with low and high myelin content. This T1 image is commonly divided by a proton-density-weighted image to generate a ratio image that is strongly T1-weighted with high intracortical contrast. Image processing tools are then applied to generate a whole-brain map of the ICM ratio signal that is projected on the middle-depth cortical surface. Various studies have confirmed these *in vivo* MRI measurements of ICM by comparing the MRI maps to histological samples of nonhuman primate brain tissue [22], as well as samples of post-mortem human brain tissue [23]. The results of these histological studies confirm that MRI-generated ICM maps provide an accurate representation of underlying myeloarchitecture across the cortex.

The MRI protocol developed by Bock et al. has been used in multiple studies of ICM in healthy [16,24] and clinical populations [16,24,25]. For example, it has been suggested that disruptions in ICM may partially underlie susceptibility to the development of mental health disorders such as bipolar disorder and schizophrenia [11]. Rowley et al., (2015) used MRI with high intracortical contrast in a sample of healthy adult females and female participants with bipolar disorder. They examined total cortical thickness, as well as myelinated (ICM) and

unmyelinated cortical thicknesses. The healthy participants displayed the greatest degree of myelination in the precentral gyrus, postcentral gyrus, Heschel's gyrus, posterior cingulate gyrus, as well as visual cortex, which is consistent with other research demonstrating that the human motor cortex (located on the precentral gyrus) displays the highest degree of myelinated fiber density [26]. The comparison of healthy participants to those with bipolar disorder revealed that total cortical thickness was significantly reduced in the bipolar disorder group, and that there was a trend towards less cortical myelination in the bipolar disorder group [16]. Additional research examining white matter in bipolar disorder has found that patients have fewer oligodendrocytes, reduced myelin-related genes, and significant age-related deficits in ICM development [27–30]. Moreover, impairments in neurocognitive performance (i.e., verbal memory) have been associated with ICM in patients with bipolar disorder [25]. In the context of schizophrenia, ICM has been shown dysregulated in the frontal lobes [31]. More recently, Tishler et al. [32] found that patients with schizophrenia displayed a significantly different age-related ICM trajectory compared to healthy controls.

The studies reviewed above suggest that ICM may be an important neural marker of psychiatric disorders; however there has yet to be a study examining ICM in addictive disorders even though there is evidence to suggest that myelin and ICM are inherently sensitive and vulnerable to neurotoxins, such as alcohol [14]. One study found that mice subjected to maternal binge-drinking in utero, exhibited reductions in multiple myelin-related proteins that were associated with various neurocognitive impairments (motor coordination, spatial awareness, etc.) [33]. As mentioned previously, there is a variety of evidence demonstrating cortical thickness and brain volume deficits in AUD, but these studies have largely examined indices of total cortical thickness or gray matter volume which do not take into account the rich neurobiology of the cortex. Fortunately, the ability to image ICM-related signal *in vivo* with the optimized MRI pulse sequences described above affords a unique opportunity to explore

differential effects in cortical sub-units. This research may elucidate whether the overall cortical reductions and disruptions are attributable to its myelin content.

The current study examined ICM in adults with AUD compared to social drinkers without AUD using the Bock et al. [21] optimized MRI protocol for imaging ICM *in vivo* and a post-processing protocol for investigating changes over the cortical surface [34]. Being the first study to examine ICM in relation to AUD, we refrained from composing a directional hypothesis and instead assumed that there would be some degree of change in ICM signal between the two groups. We also examined continuous associations between ICM and alcohol problem severity as assessed by the Alcohol Use Disorders Identification Test (AUDIT) questionnaire [35]. We selected nine bilateral *a priori* regions of interest (ROIs) based on theoretical models implicating frontal lobe circuitry [36–38], cortical thickness findings from previous AUD studies [3,4,8,39–41], and studies of neurocognitive correlates of ICM [18,19]. The ROIs examined included: middle frontal gyrus (MFG), superior frontal gyrus (SFG), inferior frontal gyrus (IFG), anterior cingulate (ACC), posterior cingulate (PCC), anterior insula, superior temporal gyrus (STG), middle temporal gyrus (MTG), and precuneus, orbital frontal gyrus (OFG), and dorsolateral prefrontal cortex (DLPFC). A whole-brain vertex-wise analysis examined effect size differences outside of the *a priori* ROIs. An exploratory aim of the current study was to examine ICM differences between male and female participants. A number of studies have identified differences between males and females with addictive disorders in relation to epidemiology, treatment outcomes, and effects on brain structure and function (for review see McHugh, Votaw, Sugarman, & Greenfield, 2018). Significant sex disparity still exists in structural brain imaging studies, with many studies not routinely reporting sex differences.

Materials and Methods

Participants

Participants were recruited from the Hamilton, Ontario, Canada community including four addiction treatment centers in the region. All participants had to be between the ages of 25-55,

have no contraindications against MRI, no history of severe head or brain trauma with loss of consciousness, neurological disorder, or severe psychiatric disorder (schizophrenia, bipolar disorder, post-traumatic stress disorder), and meet criteria for current AUD or non-hazardous social drinking. Specifically, participants in the AUD group ($N=30$) met DSM-5 criteria for AUD and did not meet DSM-5 for any substance use disorder other than nicotine dependence. Participants in the social drinker (control) group ($N=33$) reported drinking on at least a weekly basis, but did not exceed NIAAA weekly drinking limits of $\geq 14/7$ drinks per week for males/females and did not meet DSM-5 criteria for AUD or SUD, except for nicotine dependence. For full demographics, please see Table 1.

Procedures

The Hamilton Integrated Research Ethics Board approved the study (Project #1747) and participants provided informed consent. Individuals who were interested in participating were first screened to determine their eligibility. Individuals who met all eligibility criteria were then scheduled for the first of two in-person sessions. The first session took place at our laboratory or at the participant's respective treatment centers. During the first session, participants completed a battery of self-report questionnaires, a clinical interview, and neurocognitive measures. Upon completion of the first session, participants received a \$25 gift card to local stores. Those who were eligible and interested in continuing were then scheduled for an MRI at the Imaging Research Centre at St. Joseph's Healthcare Hamilton. All participants completed MRI safety screening with the MRI technologist. The MRI scan lasted roughly 40 minutes, after which participants were debriefed, received a \$25 gift card, and the session concluded.

Measures

Participants completed a variety of clinical and individual differences measures during the first session. These measures were administered either on the computer, one-on-one with a researcher, or individually using paper and pencil forms.

Structured Clinical Interview DSM-5 (SCID-5)

Diagnoses of current (i.e., in last 12 months) alcohol use disorder and substance use disorder were obtained via the Structured Clinical Interview for DSM-5 (SCID-5; First, Williams, Karg, & Spitzer, 2015). All participants completed the alcohol use disorder module and participants who reported using drugs also completed the substance use disorder module for their most often used substance.

Alcohol-related Problems

The AUDIT [35] was used as a self-report measure of drinking behavior and alcohol-related problems. The AUDIT is a 10-item self-report scale with total scores ranging from 0-40. Scores of 8 or greater are typically considered to be indicative of hazardous alcohol drinking.

Alcohol and Substance Use Frequency

The timeline follow-back interview was used to assess participants' daily alcohol consumption on for the thirty days prior to the assessment [44]. The primary dependent measure was the number of drinks consumed per week, however, given that 27 of 30 participants in the AUD group were recruited from treatment centers, there was a high percentage of participants who reported no drinking in the last 30 days. Thus, the drinks per week variable was for descriptive purposes only and not used in the primary analyses. The modified version of the National Institute on Drug Abuse Alcohol, Smoking and Substance Involvement Screening Test (NIDA Modified ASSIST, <https://www.drugabuse.gov/sites/default/files/pdf/nmassist.pdf>) assessed frequency of use of ten substances (cannabis, cocaine, prescription stimulants, methamphetamine, inhalants, sedatives, hallucinogens, street opioids, and prescription opioids). Participants rated their use in the past three months, with responses ranging from Never to Multiple Times Daily.

Nicotine Dependence

Participants who reported current smoking completed the Fagerström Test for Nicotine Dependence (FTND; [45], a validated self-report assessment of cigarette consumption and nicotine dependence severity.

Demographics

A demographic questionnaire asked participants to provide information on their age, sex assigned at birth, ethnicity, years of education, smoking status, income, and handedness.

Imaging Methods

Image Acquisition

MRI images were acquired on a 3T General Electric Discovery scanner using a 32-channel receive-only radio frequency coil for the head and a transmit radio frequency body coil. All images were acquired with 1mm isotropic resolution using a scan protocol developed by Bock and colleagues [21] and used in several prior studies of ICM [16,24,25]. Full details of the MRI scanning protocol are provided in Supplementary Materials. The total scanning protocol was roughly 40 minutes.

Image Preprocessing and Data Analysis

Image processing was performed predominately in FSL (v6.0.0), Freesurfer (v5.3.0) and Connectome Workbench (Human Connectome Project, v1.3.2). Preprocessing and ICM quantification closely followed published procedures [16,24,34]. The complete preprocessing pipeline is described in Supplementary Materials. Briefly, preprocessing included motion correction, registration to MNI space, spatial smoothing (5x5x5mm 3D median filter). A strongly T_1 -weighted ratio image was created by dividing the T_1 -weighted high intracortical contrast image by the filtered proton-density-weighted image. This removed radiofrequency receive field (B_1^-) inhomogeneities, some radiofrequency transmit field (B_1^+) inhomogeneities, and T_2^* -weighting arising from the gradient echo readout in the inversion-recovery image. Freesurfer segmentation was performed using *recon-all*. The remaining processing steps closely followed the Human Connectome Project's minimal processing pipeline using custom scripts for myelin

mapping adapted from the HCP scripts [34]. Each participant's ratio image signal is projected onto the $\frac{1}{2}$ cortical depth surface (HCP midthickness surface) using Matlab's SurfStat toolbox.

Group comparisons were performed using an *a priori* anatomical ROI approach and a whole-brain vertex-wise analysis. ROIs were generated using the multi-modal parcellation atlas (MMP) [46]. To reduce inflation of type-1 error due to many small regions, we consolidated neighboring ROIs into composite regions corresponding to our *a priori* ROIs. The 40 ROIs examined (20 symmetrical regions per hemisphere) are provided in Table 2 and anatomical locations are visualized in Figure 1 (see Supplementary Table 1 for MMP region numbers).

The mean ICM signal within each ROI was extracted and exported to SPSS Version 26 for group analyses using analysis of covariance models. Covariates were selected based on a two-step process. Demographic and substance use variables that differed significantly between groups were subsequently examined in bivariate correlations with overall ICM-related ratio signal (sum of 20 ROIs per hemisphere). Only covariates that were significantly correlated at $p < .05$ were included. An exploratory vertex-wise analysis was conducted by calculating effect size (Cohen's d) for differences between groups at each vertex. Finally, sex differences were explored by repeating the primary group comparisons including sex as a between-subjects factor. Given that this is the first study to characterize ICM in AUD, we elected to be comprehensive and report statistical significance at $p < .05$.

Results

Sample Characteristics

The AUD group ($N=30$) did not differ significantly from the control group ($N=33$) with regard to sex, age, race, or handedness (Table 1). By nature of the case-control design, the AUD group had a higher AUDIT score and drinks per week. Compared to the AUD group, the control group reported higher education and higher median income. Finally, a greater percentage of participants in the AUD group reported weekly use of cannabis (although no participants met DSM-5 criteria for cannabis use disorder) or being a current cigarette smoker.

ICM Group Maps

Surface visualization maps depicting ICM-related ratio signal for both the AUD and the control group are shown in Figure 2, Panels A-B. The pattern of anatomical distribution of ICM across groups was generally similar with the highest concentrations present throughout the visual cortex, primary motor and primary sensory regions, medial regions such as the anterior cingulate cortex (ACC), and the temporal pole. Visual comparison of the AUD and control maps suggests greater ICM-related MRI signal in the AUD group (indicated by darker or more extensive red areas in the maps), particularly in medial frontal regions in the left hemisphere, the cingulate gyrus in both hemispheres, and the anterior temporal lobe in the right hemisphere.

Region of Interest Analysis

Mean ICM-related ratio signal was extracted from the 40 *a priori* ROIs and group differences were examined using ANOVA. Although there were significant differences in a number of potential covariates (see Table 1), none of these variables were significantly correlated with overall ICM in left or right hemisphere ($r_s < .15$) although the correlation between smoking status and right hemisphere ICM was marginally significant ($r = .25$, $p = .053$). Thus, we elected to conduct the primary analyses with no covariates and then examine whether covarying for smoking impacted the statistical results.

The AUD group exhibited significantly increased ICM-related ratio signal in ten regions (see Figure 3 for ICM signal by group and Table 3 for statistical results). These included precuneus, ventromedial prefrontal cortex, posterior cingulate, and middle ACC in the left hemisphere; and middle/posterior insula, dorsolateral prefrontal cortex (Brodmann areas 8 and 46), posterior cingulate, temporal pole, and primary motor cortex in the right hemisphere. Of note, effect sizes (Cohen's d) for these significant group differences ranged from .50-.75, reflecting medium magnitude effects. When smoking was covaried, four of these effects remained significant, including precuneus, ventromedial prefrontal cortex and posterior cingulate in the left hemisphere and dorsolateral prefrontal cortex (BA8) in the right hemisphere.

Exploratory Whole-Brain Analysis

We also characterized group differences outside of the *a priori* regions of interest (ROIs) by generating a whole-brain vertex-wise map of effect sizes (Cohen's *d*) (See Figure 2, Panel C). Here, the largest effect sizes (*d*'s > .70) were located in the temporal pole, the ACC, and various medial and lateral frontal regions.

Associations with Alcohol Problem Severity

Bivariate correlations were conducted to examine whether ICM-related ratio signal was significantly associated with alcohol problem severity as assessed by the AUDIT total score. Significant positive correlations between greater ICM-related signal and greater alcohol problems were found for precuneus and middle ACC in the left hemisphere, and dorsolateral prefrontal cortex (BA8), posterior cingulate, temporal pole, and primary motor cortex in the right hemisphere.

Sex Differences in ICM

Sex differences in ICM-related MRI signal were examined by repeating the primary ANOVA models including sex at birth as an additional between-subjects factor. These analyses did not reveal any significant main effects of Sex (*ps* > .08) or Sex × Group interactions (*ps* > .11). Thus, the group differences observed above do not appear to be significantly impacted by participant sex.

Discussion

The current study is the first to use an optimized MRI pulse sequence to characterize ICM in a sample of participants with AUD and examine potential differences from a sample of healthy social drinkers. Previous research in healthy samples has reported the distribution of ICM across the cortex and provided initial clues about its role in neurocognitive processing. Prior studies with clinical samples have also indicated significant ICM disruption in people with bipolar disorder [16,24,25], schizophrenia [31,32], and other major mental health disorders [47].

Despite the increasing use of optimized MRI methods (e.g., [21]), the role of ICM in the pathophysiology of AUD remains unclear. Our results indicated that global distribution of ICM appears to be generally similar between people with AUD and controls; however, a finer-grained analysis among specific ROIs reveals significant differences between groups with small-to-medium effect size magnitudes. In all cases, the AUD group exhibited significantly greater ICM-related ratio signal compared to controls. These initial results provide important proof-of-concept for mapping ICM *in vivo* in people with AUD and, more importantly, offer a foundation for several future directions.

The primary finding was although ICM does not appear to be globally disrupted in the AUD group, it does appear to differ in specific ROIs. Comparing the two groups, we found significantly higher ICM-related ratio signal in ten regions, including DLPFC, VMPFC, PCC, ACC, posterior insula, and precuneus, among others. When covarying for smoking status, differences in DLPFC, VMPFC, precuneus, and PCC remained significant. Another important finding was the small magnitude positive correlations between higher AUDIT score and greater ICM-related signal in DLPFC, ACC, PCC, precuneus, temporal pole, among other regions. Although we refrained from constructing hypotheses related to directionality, the finding that the AUD group had *more* ICM signal than the control participants was somewhat unexpected. Unfortunately, the cross-sectional design of this study and the relatively large number of participants who were currently engaged in alcohol treatment limit our ability to make inferences about why ICM was increased or whether differences would remain in participants who were not actively trying to reduce their drinking. Nevertheless, comparing the present results with previous ICM studies in healthy and clinical populations reveals some overlap in key regions. The PCC region in the current study is in the same general location as the Grydeland et al. study in healthy participants that reported associations between ICM in PCC and error processing[18]. Exploring whether increased ICM in PCC in people with AUD is related to

neurocognitive performance is an important next step. Differences in DLPFC / Brodmann Area 46 have also been reported in previous studies with patients with bipolar disorder [25].

One potential explanation for our somewhat counterintuitive results may be that the increase in ICM signal is a compensatory response to neurotoxic effects or inflammatory responses to extended and excessive alcohol consumption. Alcohol has neurotoxic and inflammatory effects in both humans [48,49] and rodents [50]. As well, neuroinflammation can lead to the initiation of myelin repair [51,52], and oligodendrocyte precursor cells may differentiate into remyelinating oligodendrocytes when demyelination is present in the case of an injury or lesion [53]. Additionally, the byproducts of neuroinflammation, such as loss of tissue, demyelination, swelling (i.e., edema), can lead to various changes in T1 relaxation times [54] which may also be a possible explanation for the unexpected elevation in of ICM signal in the AUD group relative to controls. Importantly, much of this work is at the cellular level in animal models and should be interpreted with caution when translating findings to humans. However, it is reasonable to speculate that the increased ICM-related signal may stem from myelin-related compensatory mechanisms. As noted below, a priority for the future is to examine changes in ICM over time in people with AUD, while examining other systemic and central markers of inflammation (e.g., [55,56]).

Other priorities for future research include studying a larger sample of participants with a full range of alcohol involvement (e.g., across the continuum from social drinking through mild to severe AUD). As a proof-of-concept study, it was intentional to compare moderate/severe AUD with social drinkers, but a dimensional study will help to further characterize ICM variation in relation to AUD severity. Another important next step is to observe changes in ICM longitudinally which would not only address the question of when ICM-related differences emerge, but also what are the factors that relate to these differences such as timing of initiation of excessive drinking. Due to our cross-sectional design, we are unable to answer many compelling questions, such as whether ICM differences result from excessive alcohol use over

time or whether ICM differences are present prior to onset of AUD (i.e., consequence or cause). Another important priority is to examine what happens to ICM when people reduce or stop alcohol consumption following treatment and whether individual differences in ICM at entry to treatment predict subsequent treatment outcomes (e.g., [3,57]).

Other limitations of our current study apart from the cross-sectional design include differences between the AUD and control participants; there were some differences between groups that should lead to some caution while interpreting the results. While some differences were deliberately due to the design (e.g., AUDIT score), there were significant differences in education, income, and proportion of participants who reported tobacco or cannabis use. Of these variables, only current smoking was associated with ICM and sensitivity analyses revealed that several differences remained even after controlling for smoking status.

In conclusion, this is the first study to our knowledge to examine ICM-related MRI signal in people diagnosed with AUD. The distribution of ICM across the cortex was largely similar between the AUD and control groups, although a more precise analysis revealed several areas of greater ICM signal in the AUD participants. These initial findings offer proof-of-concept for studying ICM in addiction samples and provide a foundation for future studies to unpack the clinical and neurocognitive significance of the differences observed, neurobiological mechanisms (e.g., compensatory or neuroinflammatory changes), and potential for normalization of ICM following treatment.

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Author Contributions

MA, NB, LM, and JM were responsible for study concept and design. VM collected data with oversight from MA. VM and MA conducted the data analyses with input and interpretation from NB and JM. VM and MA wrote the first draft of the manuscript. All authors read and provided edits to the manuscript and approved the final version for submission.

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Figure Legends

Figure 1. Anatomical locations of *a priori* regions of interest generated from the multimodal parcellation atlas. A total of 20 ROIs were examined in each hemisphere. Region numbers correspond to numeric labels in Table 2, and full list of MMP regions comprising each ROI are provided in Supplementary Table 1.

Figure 2. Group average ICM maps for AUD (top) and control (middle) participants. Bottom panel depicts vertex-wise effect size maps (Cohen's d) reflecting differences between AUD and controls. In each panel data are projected onto the middle-depth cortical surface, shown in lateral, medial and superior views for left and right hemispheres separately.

Figure 3. Extracted ICM-related ratio signal values for each *a priori* ROI for left hemisphere (Panel A) and right hemisphere (Panel B). AUD group shown in black; control group shown in gray. Bars reflect mean + 1 standard error. * $p < .05$. Region abbreviations provided in Table 2.

Table 1. Sample Characteristics

Variable	AUD (N = 30)	CON (N = 33)	p
Age	39.63 (9.65)	36.93 (10.46)	.29
Sex	33% Female	58% Female	.06
Race	86% White	84% White	.67
Education	14.43 (2.62)	17.45 (2.58)	<.001
Income (Median)	\$30-45,000	\$60-75,000	<.001
DSM-5 AUD Severity	3% Moderate 97% Severe	N/A	
AUDIT	31.36 (5.50)	3.84 (1.75)	<.001
Drinks/Week	16.06 (24.35)	3.06 (1.98)	.003
Weekly Cannabis	23%	6%	.007
Current Smoker	50%	6%	<.001
FTND Total (smokers)	4.53 (2.67)	2.33 (2.08)	.20
Handedness	80% Right	84% Right	.90

Note: AUD = alcohol use disorder; CON = control; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; AUDIT = Alcohol Use Disorders Identification Test; FTND = Fagerstrom Test for Nicotine Dependence

Table 2. Region of Interest Analysis and Associations with Alcohol Severity

#	Region	Left Hemisphere				Right Hemisphere				AUDIT Total	
		<i>F</i>	<i>P</i>	ηp^2	<i>d</i>	<i>F</i>	<i>P</i>	ηp^2	<i>d</i>	Left <i>r</i>	Right <i>r</i>
1	Anterior Insula	2.50	0.119	0.039	0.40	3.83	0.055	0.059	0.49	.223	.224
2	Mid-Posterior Insula	2.32	0.133	0.037	0.38	3.99	0.049	0.061	0.50	.222	.225
3	Precuneus	4.66	0.035	0.071	0.54	2.65	0.108	0.042	0.41	.260*	.204
4	Primary Motor (M1)	1.15	0.288	0.019	0.27	5.59	0.021	0.084	0.59	.159	.329**
5	IFG	2.56	0.115	0.040	0.40	1.36	0.249	0.022	0.29	.203	.156
6	DLPFC (BA8)	3.65	0.061	0.057	0.48	8.88	0.004	0.127	0.75	.238	.341**
7	DLPFC (BA9)	2.98	0.090	0.047	0.43	3.36	0.072	0.052	0.46	.220	.207
8	DLPFC (BA46)	3.16	0.080	0.049	0.45	4.25	0.044	0.065	0.52	.234	.225
9	Medial PFC (BA10)	0.74	0.394	0.012	0.21	0.80	0.373	0.013	0.22	.114	.150
10	Medial PFC (BA8)	3.23	0.077	0.050	0.45	1.57	0.216	0.025	0.31	.201	.190
11	Medial PFC (BA9)	2.20	0.143	0.035	0.37	1.22	0.274	0.020	0.28	.186	.151
12	VMPFC	4.27	0.043	0.065	0.52	2.60	0.112	0.041	0.40	.235	.219
13	PCC (BA23)	0.71	0.403	0.012	0.21	0.26	0.610	0.004	0.13	.102	.066
14	PCC (BA31)	4.02	0.049	0.062	0.50	5.28	0.025	0.080	0.58	.241	.290*
15	Middle Cingulate	4.27	0.043	0.065	0.52	3.09	0.084	0.048	0.44	.251*	.226
16	ACC	3.40	0.070	0.053	0.46	2.63	0.110	0.041	0.41	.229	.210
17	STG	1.28	0.262	0.021	0.28	3.25	0.077	0.051	0.45	.156	.235
18	MTG	0.55	0.461	0.009	0.19	3.22	0.078	0.050	0.45	.129	.226
19	ITG	0.36	0.552	0.006	0.15	0.95	0.334	0.015	0.24	.092	.119
20	Temporal Pole	2.74	0.103	0.043	0.41	6.01	0.017	0.090	0.61	.244	.285*

Note: * $p < .05$; ** $p < .01$; IFG = inferior frontal gyrus; DLPFC = dorsolateral prefrontal cortex; PFC = prefrontal cortex; BA = Brodmann area; VMPFC = ventromedial prefrontal cortex; PCC = posterior cingulate cortex; ACC = anterior cingulate cortex; STG = superior temporal gyrus; MTG = middle temporal gyrus; ITG = inferior temporal gyrus; AUDIT = Alcohol Use Disorders Identification Test

Figure 1.

Figure 2.

Figure 3.

A) Left Hemisphere

B) Right Hemisphere

Supplementary Materials

Supplementary Methods

MRI Scanning Protocol

For our anatomical reference, we acquired a 3D T1-weighted anatomical image of the whole head using a 3D inversion-recovery gradient-echo sequence (GE 3D BRAVO; Inversion time (TI)=450ms, TE=3.2ms, TR=58.4ms, flip angle=12°, field of view (FOV)=25.6×25.6×25.6cm). This image was used as a reference for all registration steps. We also acquired two T1-weighted images with high intracortical contrast (one per hemisphere) using an inversion-recovery gradient-echo sequence (GE 3D BRAVO; TI=1000ms; TR=8.4ms; TE=3.2ms; flip angle=12°; FOV=24×10×24cm; Matrix=240×100×240cm; time between end of acquisition block and next 180° pulse (TD)=1100ms; centric phase encoding) that was used in the creation of the ICM maps. Each hemisphere was imaged separately, and the resulting images were stitched together to form a complete anatomical image. Lastly, we collected a 3D proton density-weighted image of the whole head (GE 3D FSPGR; TR=7.9ms, TE=3.1ms, flip angle=4°, FOV=24×17×24cm) to normalize intensity inhomogeneities and remove T2*-weighting in the high intracortical contrast T1-weighted image.

Image Preprocessing and ICM Calculations

Raw DICOM image files were assembled into NIFTI datasets using MRICroGL's *dcm2nii*. To account for head motion between scans, all images were registered to the T₁-weighted image with high intracortical contrast using a 6-parameter linear affine registration using FLIRT. All images (T₁, T₁ with high intracortical contrast, proton density-weighted) were then registered to the MNI 152 T₁ 1mm brain atlas. The resulting image was filtered using a 5x5x5 mm 3D median filter. A strongly T₁-weighted ratio image was created by dividing the T₁-weighted high intracortical contrast image by the filtered proton-density-weighted image. This removed radiofrequency receive field (B₁-) inhomogeneities, some radiofrequency transmit field (B₁+) inhomogeneities, and T₂*-weighting arising from the gradient echo readout in the inversion-

recovery image. The ratio image still contained some B_1+ inhomogeneity from the magnetization preparation portion of the inversion-recovery pulse sequence. Freesurfer segmentation was performed using *recon-all*, with manual corrections for gray matter / pial boundary errors. The remaining processing steps closely followed the Human Connectome Project's minimal processing pipeline using custom scripts for myelin mapping adapted from the HCP scripts [34] (<https://github.com/Washington-University/HCPpipelines>). These scripts use the output of Freesurfer's *recon-all* to generate myelin maps from the image data. Surface data was then brought into MATLAB using gifti tool box and ICM surface visualizations were created using the SurfStat toolbox.

Supplementary Table 1. Regions of Interest from Multimodal Parcellation Atlas

#	Region	MMP #s
1	Insula (anterior)	109,111,112
2	Insula (middle/posterior)	106,167,168
3	Precuneus	27
4	Primary motor cortex (M1)	8
5	Inferior frontal gyrus / orbitofrontal cortex	66,75,76,77,90, 91,92,93,94,166
6	DLPFC (BA8)	67,68,70,73
7	DLPFC (BA9)	71,87
8	DLPFC (BA46)	83,84,85,86
9	Medial frontal gyrus (BA10)	65,88
10	Medial frontal gyrus (BA8)	63
11	Medial frontal gyrus (BA9)	69
12	Ventromedial PFC	164
13	Posterior cingulate (BA23)	32,33,34
14	Posterior cingulate (BA31)	35,161,162
15	Anterior / Middle cingulate	41,57,58,59,60, 62,179,180
16	Anterior cingulate	61,64,165
17	Superior temporal	123,125,172,28
18	Middle temporal	126,128,129,130,139,176
19	Inferior temporal	132,133,134,137,177
20	Temporal pole	131,172