EXPLORING NOVEL NEUROANATOMICAL BIOMARKERS FOR ALCOHOL USE DISORDER: CONSIDERATIONS OF HIPPOCAMPAL AND AMYGDALAR SUBREGIONS, SULCAL MORPHOLOGY, AND FRACTAL DIMENSIONALITY

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Lay Abstract

Alcohol use disorder (AUD) is a disorder recognisable by continued alcohol use despite negative consequences, such as drinking leading to dangerous situations, problems in relationships, and health problems. Identifying landmarks in the brain that are different in people with AUD can help guide diagnosis and treatment. It may also help identify specific features of the brain that lead to some people having a higher risk of developing AUD. The current study employed magnetic resonance imaging (MRI) to compare structural markers in the brains of people with AUD and people who drink at healthy levels. Further, to see whether certain symptoms were more related to certain structural markers, the study looked at the relationships between each marker and each symptom used to diagnose AUD. Additionally, the study investigated whether any structural differences could be used as an effective tool to help diagnose the condition. Results of the study indicate that people with AUD have lower volume, deeper and wider folds, and less structural complexity in certain areas of their brains compared to healthy drinkers. Structural markers that differed between AUDs and healthy drinkers were related to some symptoms but not others, showing that some changes in the brain may lead to specific symptoms. Finally, certain structural markers showed potential to aid in AUD diagnosis but did not show high enough accuracy. Taken together, these findings show that certain features of the brain are different in people with AUD but that further research is needed to understand better how they relate to specific symptoms and for them to be used as diagnostic tools.

ABSTRACT

Objective: Alcohol use disorder (AUD) remains a leading cause of worldwide mortality and morbidity. The development of neuroanatomical biomarkers offers the potential of novel clinical indicators to guide prevention, early diagnosis, and treatment.

Methods: In 76 participants with DSM-5 diagnosed AUD (M_{age} = 35.75; 51.3% female) and 79 controls (M_{age} = 34.71; 59.5% female), we utilized magnetic resonance imaging (MRI) to investigate four novel measures: hippocampal and amygdalar subregion volumes, sulcal morphology (SM), and fractal dimensionality (FD). MRI processing, segmentation, and SM and FD quantification were completed using FreeSurfer v6.0 and v7.0, and MATLAB toolboxes, respectively. A significance value of $p < .05$ was employed for analysis and sex, age, and intracranial volume were included as covariates.

Results: Volumes of the right presubiculum, subiculum, and molecular layer head; left lateral and accessory basal nuclei; and corticoamygdaloid transition area were significantly lower in AUD participants relative to healthy controls. Widths of the left occipito-temporal, right middle occipital and lunate, and right marginal part of the cingulate sulci and depth of the post-central sulci were significantly increased in AUD participants relative to controls. Finally, decreased left caudate, left thalamus, right putamen and right pallidum FD and greater inferior lateral and third ventricle FD were observed in AUD participants relative to controls. Each novel measure's reliability was assessed using test-retest data from the Human Connectome Project and indicated high reliability with median intraclass correlations of .93, .91, .88, and .93 for the hippocampal subfields, amygdalar nuclei, SM, and FD, respectively.

Conclusion: These results indicate selectively decreased hippocampal and amygdala subregion volume, increased sulcal depth and width, and differences in FD as promising neuroanatomical biomarkers for AUD.

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List of Abbreviations

ACC: Anterior Cingulate Cortex

AD: Alzheimer's Disease

AUD: Alcohol Use Disorder

CA: Cornu Ammonis

CT: Cortical Thickness

CTRL: Controls

DART: Diagnostic Assessment Research Tool

DKT: Desikan–Killiany–Tourville

DG: Dentate Gyrus

DSM-5: Diagnostic Statistical Manual of Mental Disorders, 5th Edition

FA: Flip Angle

FD: Fractal Dimensionality

FOV: Field of View

HATA: Hippocampal Amygdaloid Transition Area

ICM: Intracortical Myelin

ICC: Intraclass Correlation Coefficient

GMV: Gray Matter Volume

M: Mean

MRI: Magnetic Resonance Imaging

OFC: Orbitofrontal Cortex

SA: Surface Area

SE: Standard Error

SM: Sulcal Morphology

ROI: Region of Interest

SCID: Structured Clinical Interview for DSM-5

SPSS: Statistical Package for the Social Sciences

TE: Echo Time

TLFB: Timeline Follow Back

TR: Repetition Time

V: Volume

VBM: Voxel Based Morphometry

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CHAPTER 1: INTRODUCTION AND OVERVIEW

1.1 Alcohol Use Disorder

Alcohol is a widely used psychoactive and neurotoxic substance that, while ingrained into societal norms, can be extremely destructive for select individuals, their friends and families, and communities as a whole. Worldwide, alcohol use is a leading cause of mortality, accounting for 7.2% of all premature deaths in 2016, with rates elevated to 13.5% in those aged 20-39 years (World Health Organization, 2018). Leading causes of alcohol-attributable deaths include injury, digestive, cardiovascular and infectious diseases, and cancers (World Health Organization, 2018). Alcohol use disorder (AUD) is a chronic, clinically diagnosed condition defined by an inability to moderate alcohol use despite adverse social, physical, psychological, and occupational consequences (National Institute of Alcohol Abuse and Alcoholism [NIAAA], 2021). In the United States alone, the lifetime prevalence of AUD in those 18 years and older is 29.1% of the population, or 68,485,000 individuals (Grant et al., 2017). Once developed, recovery can be highly challenging, and for many, developing healthy drinking levels may be impossible, leaving sobriety as their only option and relapse common (Tuithof et al., 2014).

1.2 Etiology and Diagnosis

The etiology of AUD is complex and involves a combination of many biopsychosocial factors. Accordingly, susceptibility to AUD can vary greatly and includes risk factors that range from the individual level to the environmental level. On the individual level, risk factors include age (Fink et al., 2016), sex (Flores-Bonilla &

Richardson, 2020; Goldstick et al., 2019), genetics (Verhulst et al., 2015), family history (Spadoni et al., 2013; Chassin et al., 2004), educational attainment and achievement (Kendler et al., 2017; Englung et al., 2008), socioeconomic status (Calling et al., 2019), personality traits (e.g., externalizing and internalizing behaviours; Englung et al., 2008; Sintov et al., 2009), and structural and functional features of the brain (Dupuy & Chanraud, 2016; Fauth-Bühler & Mann, 2011). On the social level, risk factors include peer groups (Steinberg et al., 1994), familial relations (King et al., 2009), and social norms (Lee et al., 2010). And, on the macro-environmental level, risk factors include alcohol availability (Chen et al., 2010; Bjarnason et al., 2003) and urbanicity (Dixon & Chartier, 2016).

Given the heterogeneity of etiological factors and symptoms associated with AUD, it has been theorized that up to five subtypes exist (Leggio et al., 2009). Within these proposed subtypes, commonalities can be noted. For example, binary models by Cloninger et al. (1981) and Babor et al. (1992) include common subtypes: 1) Subtypes A and I both possess traits reflecting later onset, fewer alcohol social and physical consequences, and less treatment, and 2) Subtypes B and II both possess traits such reflecting earlier onset, hazardous use, family history, externalizing behaviours (i.e., antisocial acts), and chronic treatment. Similar to binary models, when expanding consideration to three-type, four-type, and five-type models, one AUD subtype often remains rooted in externalizing behaviour (e.g., impulsive or aggressive behaviour). These include Type II from Hill (1992), Externalizing Type from Del Boca & Hesselbrook (1996), and Chronic/ ASP from Windle & Scheidt (2004). The existence of

an AUD subtype rooted in externalizing behaviour is also lively given robust associations between externalizing behaviour and AUD (Farmer et al., 2016) and that externalizing behaviours are heritable and contribute to AUD risk (Kendler et al., 2011; Haber et al., 2005).

AUD is clinically diagnosed based on the endorsement of specific criteria, including persistent consumption despite interferences with occupational and social obligations, hazardous use, detriments to physical and mental health, and presence of withdrawal or tolerance (5th ed.; DSM-5; American Psychiatric Association, 2013). AUD severity can be classified as mild (2-3 symptoms), moderate (4-5 symptoms), or severe (6+ symptoms). Given the wide range of AUD diagnostic criteria included in the DSM-5 (see Appendix C), the possibility of AUD subtypes is logical; while some are more reflective of externalizing behaviour, such as interpersonal problems and hazardous use, others, such as tolerance and withdrawal, may be due to altered biology related to alcohol exposure (i.e., neuroadaptation). Based on the role of externalizing behaviour in AUD, McDowell et al. (2020) have arranged the AUD diagnostic criteria onto a spectrum from criteria more theoretically based in neuroadaptation to those more theoretically based in externalizing behaviour (see Table 1.1 for visual adaptation). This spectrum may be valuable to consider when exploring neuroanatomical correlates of AUD as some alterations may be more related to externalizing AUD subtypes.

Table 1. 1

List of DSM-5 AUD diagnostic criteria rearranged in order of theoretical association with externalizing behaviour

DSM-5 Criteria #	Diagnostic Criteria	Association to Externalizing Behaviour
11	Experience of Withdrawal	Less Externalizing
10	Development of Tolerance	
4	Alcohol Cravings	
7	Activities Given Up	
9	Physical/ Psychological Problems	
3	Substantial Time Devotion	
2	Unable to Quit/ Cut Down	
1	Larger/ Longer Drinking	
5	Obligation Interference	
6	Social Problems	
8	Hazardous Use	More Externalizing

Note. Table adapted from McDowell et al., (2019). See Appendix D for full complete diagnostic criteria. DSM-5 = diagnostic and statistical manual of mental disorders, 5th ed. AUD = alcohol use disorder.

1.3 Structural Correlates of AUD

1.3.1 Cortical Abnormalities

Numerous studies have substantiated the presence of cortical dysmorphology in relation to AUD, primarily through assessment of cortical gray matter volume (GMV), cortical thickness (CT), and cortical surface area (SA). While literature solidifying the relationship between global cortical gray matter volume reductions and AUD is unassailable (Momenan et al., 2012; Fein et al., 2006), there is considerable breadth and variation in local cortical alteration findings. Ambiguity in local cortical results is likely

attributable to several factors including variations in methods of analysis (e.g., voxel-based morphology [VBM], selected regions of interest [ROI], and parcellation techniques), general participant demographics (e.g., age and sex differences), and AUD participant characteristics (e.g., treatment seeking or non-treatment seeking, current drinker or abstinent). Further, given the variation in AUD etiological factors (e.g., trauma or high stress environments) and the potential of AUD subtypes (e.g., those rooted in externalizing behaviour), between-subject differences in morphological underpinnings are likely and may also be contributing to ambiguity.

Despite variations in studies, the finding of reduced volume within the frontal and parietal lobes is prevalent (Pfefferbaum et al., 1998). For example, Momenan et al. (2012) compared participants with AUD (n = 130, 36% female) and healthy controls (n = 69, 36% female) using a voxel-based approach and found locally reduced CT in frontal lobe regions (i.e., right medial frontal and precentral/inferior frontal gyri), the precuneus, and the right insula. Importantly, years of heavy drinking was only predictive of CT for the whole right hemisphere and right precentral frontal gyrus, indicating that these regions may be particularly associated with neurotoxic effects. Similarly, Fein et al. (2002) explored cortical GMV loss in treatment naive DSM-4 diagnosed alcohol dependent males (n = 24) and healthy controls (n = 17) and found reduced cortical GMV in the frontal and parietal lobes. Durazzo et al. (2011) also compared surface area and regional volume of cortical structures in DSM-5 diagnosed AUD participants (n = 75, 5% female) and healthy controls (n = 43, 9% female), with results exhibiting no significant differences in regional surface area. However, when considering regional cortical

thickness, AUD participants exhibited significantly thinner cortices within the anterior cingulate cortex (ACC; right rostral ACC), frontal cortex (bilateral rostral middle and caudle middle), bilateral insula, and orbitofrontal cortex (right medial and bilateral lateral OFC). Finally, when considering the regional volume, AUD participants exhibited significantly lower volumes in the ACC (left rostral and right caudal ACC), frontal cortex (left caudal middle, and bilateral rostral middle and superior), bilateral insula, and OFC (bilateral medial and right lateral OFC). While these findings highlight the prominence of cortical alterations throughout the frontal lobe, they also highlight the differences in methodology, sample characteristics, and breadth of current literature on cortical abnormalities relating to AUD.

1.3.2 Subcortical Abnormalities

Subcortical GMV reductions in relation to alcohol use are also prevalent in the literature (Durazzo et al., 2011; Yang et al., 2016; Dager et al., 2015). In a mega-analysis by Mackey et al. (2018) comparing alcohol-dependent individuals (n= 898, 32.4 % female) with non-alcohol dependent controls (n = 292, 33.9% female), results demonstrated significantly reduced volumes of the bilateral hippocampus, amygdala, putamen; right thalamus and globus pallidus; and left nucleus accumbens in alcohol-dependent participants relative to controls. Of note, subcortical structures do not seem to exhibit volume recovery with abstinence from alcohol to the same extent as cortical regions (van Ejik et al., 2013; Zou et al., 2018). For example, after two weeks of abstinence, Wang et al. (2015) found no volume recovery in subcortical structures but did find partial degrees of cortical thickness recovery. Consequently, it has been theorized

that subcortical GMV abnormalities are either irreversibly damaged or reflect genetic or environmental predispositions to AUD (Dager et al., 2015; Wang et al., 2015).

1.4 Potential Novel Biomarkers

1.4.1 Hippocampal Subfields

Embedded in the temporal lobe and occupying the posterior section of the limbic lobe, the hippocampus plays a vital role in learning and memory (Anand et al., 2012). It has also been implicated in emotional regulation, motivation memory, and contextual processing due to its anatomical linkages to the prefrontal cortex (PFC), amygdala, and nucleus accumbens (Ding, 2013). Structurally, the hippocampus can be segregated into three main sections: the head, the body, and the tail (Anande et al., 2012). The hippocampus appears to be particularly vulnerable to environmental stressors (Kim & Diamond, 2002), and severe hippocampal atrophy is observed in several neuropsychiatric conditions, including Alzheimer's disease (de Flores et al., 2015), depression (Bremner et al., 2000), schizophrenia (Roeske et al., 2020), and epilepsy (Anand et al., 2012; Roeske et al., 2020). Decreased hippocampal volume, and in particular focal atrophy of the cornu ammonis (CA) 1 region of the hippocampus, has become a widely accepted, accessible, and non-invasive biomarker for Alzheimer's disease, highlighting the potential for hippocampal volumetric alterations to inform the diagnosis of other neurological and psychological conditions (de Flores et al., 2015).

Hippocampal volume reductions related to problematic alcohol use have been unassailably demonstrated within several studies and appear to be more prominent in clinically diagnosed AUD than subclinical and in adults than adolescents (Wilson et al.,

2017). However, these findings fail to consider that the hippocampus is a cytoarchitecturally and histologically complex structure with distinct subfields. With the emergence of MRI acquisition techniques that allow higher resolution images, the distinct functional roles and focal volumetric alterations of hippocampal subfields can now be investigated (Van Leemput at al., 2009).

To date, three studies have considered the heterogeneous subfields of the hippocampus in relation to AUD. The first was a longitudinal study completed by Kuhn et al. in 2014 investigating volumetric changes in hippocampal subfields with alcohol abstinence. The study included 42 AUD participants and 32 healthy controls, and subfield segmentation was completed using FreeSurfer 5.2.0. The authors opted only to consider volumetric measures for CA2 and 3, CA4 and dentate gyrus (DG), and the subiculum as automatic segmentation of these larger subfields has been validated and shown to be highly correlated with manual segmentation (Van Leemput et al., 2009). Participants were scanned once after initial withdrawal from alcohol and again after 2 weeks. Results demonstrated that AUD participants had lower initial CA2+3 volumes relative to controls, which significantly normalized after 2 weeks of abstinence. Results also indicated a significant relationship between initial CA2+3 volume and years of regular alcohol consumption and maximal severity of alcohol-withdrawal score. The second study to consider hippocampal subfields in relation to AUD was completed by Lee et al. (2016) and included 26 males with DSM-5 diagnosed AUD and 26 age-matched healthy controls. Automatic segmentation of the hippocampus was achieved with T1-weighted images and using FreeSurfer 5.3. Results revealed reduced volumes in AUD participants'

left presubiculum, fimbria, and bilateral subiculum compared to controls. The final study by Zahr et al. (2019), included 24 DSM-5 diagnosed AUD participants (29.2% female) and 20 controls (35% female). Zahr et al. (2019) employed automatic hippocampal segmentation of T1- and T2-weighted images available through FreeSurfer 6.0 to analyze volumetric measures for 12 subfields of the hippocampus and revealed significantly reduced volumes of the subiculum, CA1, CA4, granule cell and molecular layer of the dentate gyrus (GC-ML-DG), hippocampus-amygdala-transition-area (HATA), and fimbria in AUD participants relative to controls. Results also revealed a diagnosis-by-age interaction of CA2+3, indicating AUD participants had volume reductions in this subfield beyond age related changes. Of note, the AUD group in the study were abstinent for the 3 months preceding the study and received pharmacological treatment. While these studies have established a foundation for dysmorphology of hippocampal subfields in relation to problematic alcohol use, the current study aims to augment findings using a large, sex balanced sample ($n_{AUD} = 76$; $n_{CTRL} = 79$) and automatic segmentation available through FreeSurfer 7.1, which offers higher resolution segmentation and further subdivision within head, body, and tail regions.

1.4.2 Nuclei of the Amygdala

Embedded in the medial temporal lobe and occupying the anterior portion of the hippocampal formation, the amygdala is a limbic structure that plays essential roles in fear-based emotion acquisition, integration, storage, and memory, fear conditioning, and behaviour control (Rajmohan & Mohandas, 2007; Le Doux, 2000; Wrase et al., 2008). Though small, the amygdala possesses numerous afferent connections (e.g.,

hypothalamus, medial PFC, brainstem, septum, sensory cortex, thalamus, and hippocampus and efferent connections (e.g., ventral pallidum, entorhinal cortex, and lateral hypothalamus) throughout the brain (Otterson, 1980; Sah et al., 2003). Global alterations in amygdala volume in relation to AUD are well established in the literature (Wrase et al., 2008; Orban et al., 2019); however, whether these volumetric reductions antedate AUD or are due to alcohol exposure is less clear. It has been theorized that amygdala volume alterations predate development of AUD as young adults with a family history of alcohol misuse have been shown to have reduced amygdala volume (Fein et al., 2006; Dager et al., 2015) and significant amygdalar volume appear to persist even with long-term abstinence (Hill et al., 2001; Dager et al., 2015).

Like the hippocampus, the amygdala has traditionally been investigated as a homogeneous entity; however, research has since revealed that it is composed of multiple nuclei with distinct functions, connectivity, and cellular features (Brown et al., 2019; Abivardi & Back, 2017). Advancements in high-resolution neuroimaging techniques now permit automatic segmentation and volumetric assessment of individual nuclei (Saygin et al., 2017). Despite ample evidence supporting the relationship between amygdala volume and AUD, research considering the volume of individual nuclei of the amygdala in relation to AUD remains very limited. To our knowledge, just one study has been performed, wherein Phillips et al. (2021) found that greater alcohol use was significantly associated with increased volume of the right basal nucleus in a sample of adolescents. This result is discordant with previous findings of global hippocampal volume reductions in individuals with AUD, given volume and alcohol use were positively correlated rather

than negative. However, given that the study was composed of adolescent participants, larger subfield volume may reflect a delay in gray matter pruning (Hill, 2018), whereas larger volume in adult samples often reflects reduced age- related or disease-related atrophy. Further research exploring volumetric alterations in amygdala nuclei in relation to alcohol use are required to clarify these findings and to permit generalization to an adult sample.

1.4.3 Sulcal Morphology

Sulcal morphology assesses the morphology of the cortex by estimating the width and depth of several major sulci of the brain. Traditionally, cortical morphology has been assessed using measurements of GMV, cortical thickness, surface area, or gyrification (Madan, 2019). Enlargement of cortical sulci has been established as a natural representation of age-related atrophy (Drayer, 1988), however, research has demonstrated that the degree of atrophy varies greatly between individuals and regions of the brain (Coffey et al., 1992). The utility of SM as a diagnostic criterion for Alzheimer's disease (AD) has also recently been demonstrated in a study by Bertoux et al., (2019), wherein they found that sulcal width was a better predictor of AD than sulcal cortical thickness, regional cortical volume, cortical thickness, and hippocampal volume. Similar results have also been found in other studies investigating the same relationship (Hamelin et al., 2015; Cai et al., 2017). Furthermore, as SM analysis is not dependent on gray and white matter contrast, it is a more resilient measure to study pathological processes in which the contrasts weaken (Bertoux et al., 2019). To date, SM measures have not been studied in relation to AUD; however, given toolboxes have only recently become available and

given its effectiveness and sensitivity as a measure, SM may offer great potential as a biomarker for AUD.

1.3.4 Fractal Dimensionality

Fractal dimensionality, originally conceived as fractional dimensionality, is a measure of the geometric complexity of irregular natural structures (e.g., coastlines, clouds, snowflakes) that cannot be assessed in standard parameters like the solid figures of conventional or Euclidian geometry (e.g., circles). While the original mathematical theories were largely developed in the late 19th and early 20th centuries, the term FD was not popularized until 1975 when by Benoît Mandelbrot coined the term based on the Latin word *fractus* meaning "fragmented" or "broken" (Mandelbrot, 1985). Etymologically, Mandelbrot also related the term to the word fraction, meaning between integers, as the fractal set of an irregular structure lies between Euclid shapes (Mandelbrot, 1985). FD has since been applied to complex biological components of the human body, including liver histopathological structures, microvasculature of histological specimens, and various aspects of the brain such as the cerebral cortex (Grizzi et al., 2021; Di leva et al., 2012; Reishofer et al., 2018; Kiselev et al., 2003). Pertaining to the brain, alterations in FD have been linked to several conditions, including significant reductions in the cortical FD of mild Alzheimer's (King et al., 2010) and acute anorexia nervosa patients (Collantoni et al., 2020), and decreased white matter FD in multiple sclerosis patients (Esteban et al., 2008). Like SM, alterations in FD have not yet been studied in the context of AUD, thus the current study aims to establish research in this area by assessing cortical, subcortical, and ventricular FD in relation to AUD.

1.5 Present Study Rationale

The underlying mechanisms which separate those able to maintain healthy drinking levels and those prone to developing AUD remain unclear. Further, current diagnosis of AUD largely relies on the administration of the DSM-5 which, despite having good to excellent inter-rater reliability (Dennis et al., 2015; Hasin et al., 2020), permits subjective interpretations, inaccurate reports from patients, and does not distinguish between potential AUD subtypes. Accordingly, establishing structural biomarkers that can conspicuously reveal AUD presence, severity, and identify subtypes would streamline diagnosis and treatment. In addition, these biomarkers may offer insight into the etiology of AUD and guide prevention efforts. Research on several neurological disorders, including Alzheimer's disease and schizophrenia, has revealed the potential and utility of structural neuroimaging measures as biomarkers and predictors of disease progression (McEvoy et al., 2009; Li et al., 2020). Although studies have used structural MRI to investigate morphological brain alterations in relation to AUD, no clear biomarkers have been identified. This may be due to a focus of existing findings on CT, cortical SA, GMV, and global volume reductions of structures (e.g., whole hippocampus or brainstem). Exploration of novel biomarkers may provide the clues needed to advance this field of research.

The overall aims of the current study were trifold. First, we aimed to clarify neurocorrelates of AUD by systematically assessing cortical and subcortical features of the brain using novel measures including volumetric analysis of hippocampal and amygdalar subfields, sulcal morphology, and fractal dimensionality. Second, we aimed to

explore the possibility of AUD subtypes in our sample based on variations in bivariate associations between novel measures that differed between groups and individual AUD diagnostic criteria. And third, we aimed to test the potential of the novel measures that differed significantly between groups to be employed as unbiased diagnostic tools. Given the incipient nature of the study, specific regional hypotheses were not formulated, rather neuroanatomical differences were postulated to be present between groups.

CHAPTER 2: METHODS

2.1 Participants

2.1.1 Inclusion and Exclusion Criteria

The current study included 155 participants recruited by means of local advertisement as part of two independent case-control studies in the local community of Hamilton, Ontario: NeuroAlc (NA) and Intracortical Myelin (ICM). All eligible participants were aged 21-55 (NA) or 25-55 (ICM) years and were required to be fluent in English. Eligible AUD participants had a current DSM-5 AUD diagnosis and reported hazardous drinking levels (i.e., females > 7 weekly drinks, males > 14 weekly drinks; U.S. Preventative Services Task Force, 2004). Eligible control participants were low-risk drinkers with no current DSM-5 diagnosis and reported alcohol consumption that did not exceed hazardous drinking levels (i.e., females ≤ 7 weekly drinks, males ≤ 14 weekly drinks; U.S. Preventative Services Task Force, 2004). Common exclusion criteria included: i) history of severe brain trauma, ii) history of neurological disorder (e.g., Parkinson's disease, multiple sclerosis), iii) history of psychiatric disorder (e.g., schizophrenia-spectrum, psychotic, bipolar disorders), MRI contraindications (e.g., pregnancy/breastfeeding, metallic implants, claustrophobia), or iv) current DSM-5 diagnosed substance use disorder other than alcohol or nicotine. NA study participants were also permitted to have a current cannabis use disorder and were required to be non-treatment seeking. Contrarily, 27 of the 30 AUD participants who were recruited through ICM were currently in treatment. For a comprehensive list of inclusion and exclusion criteria for both studies see Appendix A.

2.1.2 Sample Characteristics

As displayed in Table 1, the final sample included 76 AUD and 79 control participants. AUD participants were, on average, in their mid-thirties, had a median income of $45,000-$60,000, and were balanced between sexes (51.3% female). Control participants were, on average, in their mid-thirties, with a median income of $60,000-$75,000, and a modest overrepresentation of females (59.5% female). AUD and control participants did not differ significantly based on age, sex, race, and handedness. However, they did differ significantly based on income and years of education, with control participants having, on average, 2 years further education and having an income of approximately $15,000 higher. As a result, years of education and income were included as covariates in analysis. In accordance with the study designs, AUD and control participants differed significantly in number of AUD symptoms and drinks per week.

Comparisons of AUD participant characteristics from NA (n=46) and ICM (n = 30) and of control participant characteristics from NA (n = 30) and ICM (n = 49) are available in Appendix B. Characteristics of NA and ICM participants did not differ significantly between studies based on education, race, and handedness; however, they did differ significantly based on age, sex, income, and AUD severity, with ICM participants being more male weighted, on average 3 years older, having an income $30,000 lower, and having two further AUD symptoms. NA and ICM control participants were balanced for all characteristics apart from age, with ICM controls being, on average, 5 years older.

Table 2. 1

Aggregated sample comparison of control and AUD participant characteristics

Variable (mean [SD] / %)	**CTRL[a]**	**AUD[b]**	**Mean Difference (T-Test)**	**t**	***p*-value**	**d**
Age	34.71 [10.33]	35.75 [10.80]	-1.04	-0.61	.541	-.010
Sex (% Female)	59.5	51.3	0.08	1.02	.309	0.16
Handedness (% RH)	98.7	93.3[c]	-0.01	-0.25	.804	-0.40
Income (Median)	**$60,000 - $75,000**	**$45000 - $60000[c]**	**1.00**	**2.38**	**.019**	**0.38**
Years of Education	**16.71**	**14.62**	**2.09**	**4.38**	**2.2E-5**	**0.70**
Race (% European White)	84.8	86.7[c]	0.09	0.33	.744	0.05
# AUD symptoms	**0.03 [.16]**	**7.55 [2.50]**	**-7.53**	**-26.71**	**1.8E-59**	**-4.29**
Drinks/week	**5.13 [4.82]**	**23.00 [15.92]***	**-17.87**	**-9.29**	**6.9E-16**	**-1.72**

Note. [a]n = 79. [b]n = 76. [c]n = 75. *ICM drinking data for AUD participants is not included as they were in-treatment. Bold indicates characteristics that differed significantly between AUD and control participants.

2.2 Procedures

In both studies, interested participants first completed telephone interviews to confirm eligibility. Eligible participants were then invited to complete a baseline session

which included a battery of self-report questionnaires, neurocognitive assessments, and semi-structured diagnostic and timeline follow-back interviews to assess drinking behaviour. Those who retained eligibility based on their responses during the first assessment were scheduled for a second session that included an MRI scan at the Imaging Research Centre at St. Joseph's Healthcare Hamilton. At the beginning of each session, participant's sobriety was confirmed using a AlcoSensor Breathalyzer (prior to COVID-19 pandemic) or self-report questionnaire (post COVID-19 pandemic). Participants also supplied a urine sample from which recent drug use was assessed using a commercial urine drug screen. Written informed consent was obtained from all participants and protocols were approved by the Hamilton Integrated Research Ethics Board (NA = Project #4551; ICM = Project #1747). Participants received modest compensation for all completed sessions in the form of gift cards.

2.3 Out-of-Scanner Assessments

2.3.1 Demographics

Demographic questionnaires were completed by all participants wherein they provided information on their age, sex, race, and education.

2.3.2 Diagnostic Assessments

All participants were administered semi-structured diagnostic assessments by trained research associates to assess alcohol severity based on DSM-5 criteria. Participants in the NeuroAlc study were administered the Diagnostic Assessment Research Tool (DART; McCabe et al., 2017) and participants in the ICM study were administered the Structured Clinical Interview for DSM-5 (SCID-5; First, Williams,

Karg, & Spitzer, 2015). Both assessments aim to systematically diagnose AUD using the 11 diagnostic criteria of the DSM-5 (5th ed.; DSM-5; American Psychiatric Association, 2013). Criteria include failed attempts to cut down on alcohol use, interference in work, school, or home responsibilities, and presence of tolerance and withdrawal to alcohol (see Appendix C for a complete list). On both assessments, endorsement of three to four criteria is diagnosed as mild AUD, four to five is diagnosed as moderate AUD, and six or more is diagnosed as severe AUD.

2.3.3 Timeline Follow-Back Interview

Daily drinking levels for the 4 weeks preceding the baseline assessment were obtained from all participants using a timeline follow-back interview (TLFB) which was administered by trained research associates (Sobell & Sobell, 1992). To enhance recall, participants were shown a calendar with which they could identify key dates and estimate their daily drinking retrospectively. Participants were asked to recall what they were drinking and how many drinks they consumed. Research associates also inquired as to whether the drinks were consumed at the participant's home or a restaurant or bar-type establishment, to assess whether alcohol was served in standard drink sizes (i.e., 8oz of wine). All drinks were converted to standard drink units to permit comparison (i.e., 5oz of wine = 1 standard drink; NIAAA, 2000). TLFB responses were not valid from the ICM AUD subsample as participants were largely recruited from treatment centres, creating inaccurate estimates that would not reflect standard drinking behaviour.

2.4 Imaging Methods

2.4.1 Image Acquisition

All participants were scanned using a 3T General Electric Discovery whole-body, short bore scanner with a 32-channel head coil (General Electric, Milwaukee, WI, USA) at the Hamilton Integrated Research Centre. While both T1- and T2-weighted scans were collected, the analysis in the current study centered on whole brain anatomical images obtained from high-resolution T1-weighted images. The parameters for the T1-weighted images were as follows: D BRAVO sequence, straight axial plane, field of view (FOV) = 25.6 cm, matrix = 256 x 256, 192 slices, repetition time (TR) = 8.2 ms, echo time (TE) = 3.2 ms, inversion time (TI) = 450 ms, flip angle (FA) = 12°, slice thickness = 1mm, bandwidth (BW) = 31.25kHz (244 Hz/pixel), and acceleration factor = 2.

2.4.2 Image Preprocessing

To begin, all raw T1-weighted images were converted from DICOM to NIFTI format using SPM (SPM, version 12). Images were then preprocessed using the standard FreeSurfer (version 6.0) processing stream recon-all which has been widely used in MRI studies for preprocessing and quantification of neuroanatomical structures (Fischl, 2012; https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all). The processing stream includes a total of 31 preprocessing steps, which include motion correction, segmentation of neuroanatomical structures (Fischl et al., 2002), tessellation and removal of topological defects of the white matter surface (Dale et al., 1999), inflation of white matter surfaces (Fischl et al., 1999a), and surface-based registration to the FreeSurfer average template to increase the accuracy of alignment to major sulcal and gyral landmarks (Fischl et al.,

1999b). Finally, the processing stream culminates with sulcal and gyral segmentation (Desikan et al., 2006), cortical parcellation, and generation of parcellation statistics including volumetric and surface area measurements.

To prepare MRI data for SM and FD quantification, the pial output from the recon-all pipeline (h.pial) was processed through an additional script, Local Gyrification Index (Schaer et al., 2008; https://surfer.nmr.mgh.harvard.edu/fswiki/LGI). This processing stream creates a smoothed outer surface that tightly wraps the pial surface and uses it to create a ratio with each vertex of the pial surface within three-dimensional regions of interest. The final output files include ".pial-outer-smoothed" which were used as an input for the generation of SM and FD measurements.

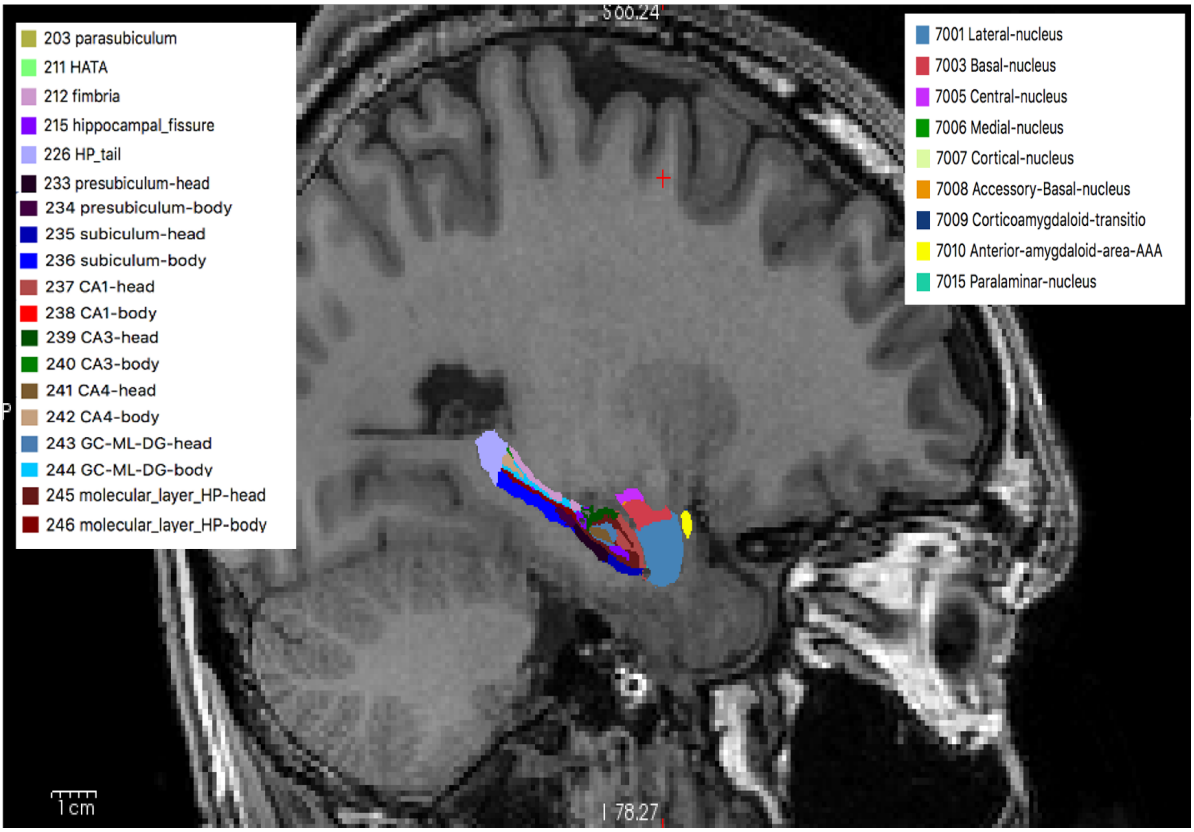
2.4.3 Hippocampal and Amygdala Subfield Segmentation

To investigate volumetric differences in subregions of the hippocampus and amygdala, processed T1-weighted images were automatically segmented using a FreeSurfer algorithm (version 7.1; https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala). Automated segmentation of the hippocampus is based on registration to probabilistic atlas derived from a combination of ultra-high resolution (i.e., on average 0.13 mm isotropic) ex vivo and high resolution (i.e., 1 mm isotropic) in vivo data which were merged into a single computational atlas using a Bayesian inference approach (Iglesias, 2015). Unlike other methods (e.g., Yushkevich et al., 2010), this module uses a fully automatic, generative, parametric approach using labeled training data that does not require any prior knowledge on distribution of image intensities (Iglesias, 2015).

Segmentation produces volumetric measures for 9 bilateral subfields within the hippocampal head (i.e., parasubiculum, presubiculum head, subiculum head, CA1 head, CA3 head, CA4 head, granule cell of the molecular layer of the dentate gyrus [GC-ML-DG] head, molecular layer head, HATA), 8 bilateral subfields within the hippocampal body (i.e., presubiculum body, subiculum body, CA1 body, CA3 body, CA4 body, GC-ML-DG body, molecular layer body, fimbria), 1 measure for the hippocampal tail, and 1 for the hippocampal fissure. Of note, the CA3 subfield volume also includes CA2. Similar to hippocampal segmentation, automatic segmentation of the nuclei of the amygdala uses a probabilistic atlas created using an algorithm based on Bayesian inference on ultra-high resolution (100-150μm at 7T) ex vivo data. Amygdalar segmentation produces volumes for 8 bilateral nuclei: lateral nucleus, basal nucleus, accessory basal nucleus, central nucleus, paralaminar nucleus, cortico-amygdaloid transition area, cortical nucleus, and medial nucleus. See Figure 2.1 for an example of hippocampal and amygdalar segmentation from a participant in the current study.

Figure 2. 1

Sagittal view displaying subfields of the hippocampus (left legend) and amygdala (right legend) in a control participant from the current study



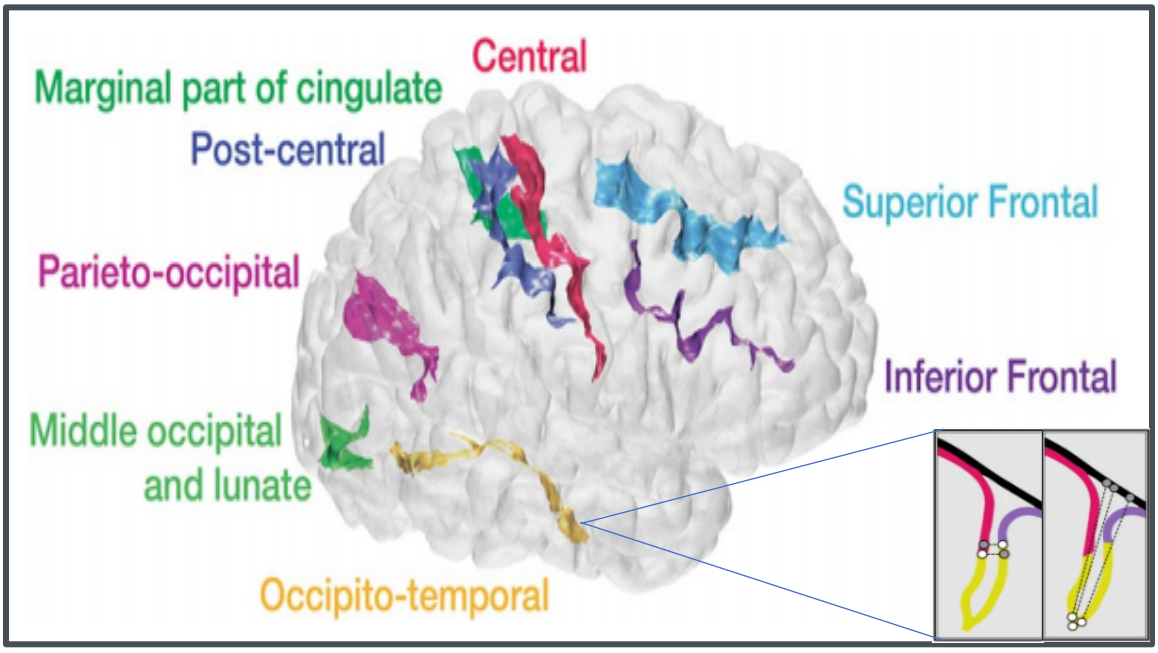
2.4.4 Sulcal Morphology

Sulcal morphology was employed as a measure to assess the width and depth of several sulci of the brain. Measurements were obtained via the validated MATLAB toolbox *calcSulc* (Madan, 2019), which bases calculations on the cortical reconstruction (?h.pial), parcellation (h.aparc.a2009.annot), and sulcal map (?h.sulc) outputs of the FreeSurfer recon-all pipeline along with the "?h.pial-outer-smoothed" output from the local gyrification analysis (Madan, 2019; https://cmadan.github.io/calcSulc/). Cortical

parcellation was based on the Destrieux et al. atlas which is included within the standard recon-all pipeline (Destrieux et al., 2010). The toolbox produces estimates of bilateral sulcal widths and depths in eight major sulci of the brain: 1) occipito-temporal, 2) middle occipital and lunate, 3) parieto-occipital, 4) post-central, 5) marginal part of the cingulate, 6) central, 7) superior frontal, and 8) inferior frontal (32 measurements total; see Figure 2 for visual representation). The width measurement is calculated by marking the vertices at the boundary between the gyrus and the sulcus on both sides and then finding the shortest distance between each boundary vertex and a vertex on the opposite side. The sulcal depth is similarly calculated by identifying vertices at the fundus of the sulcus and finding the shortest distance between each vertex of the fundus and a vertex on the enclosing surface of the sulcus.

Figure 2. 2

The eight sulci that are assessed for depth and width using the sulcal morphology toolbox



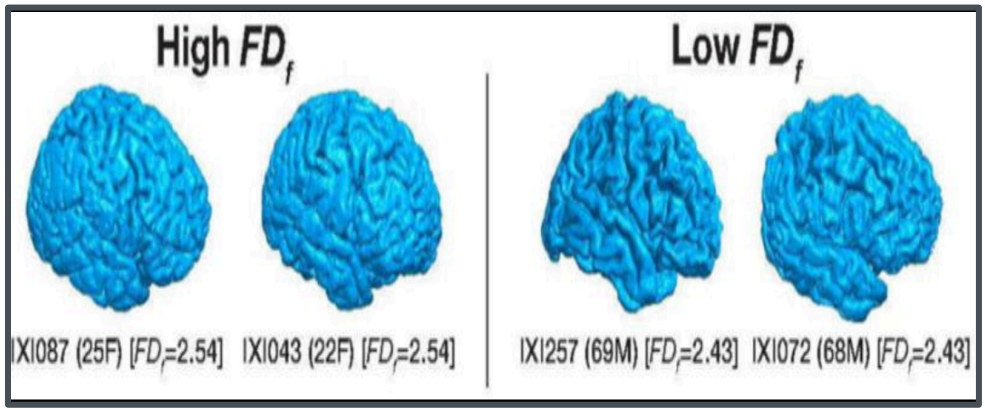
2.4.5 Fractal Dimensionality

FD was employed as a measure to assess the structural complexity of parcellated cortical regions, segmented subcortical structures, lobes, and ventricles of the brain. FD measurements were obtained via the validated MATLAB toolbox *calcFD*, which bases calculations on cortical reconstruction and parcellation outputs from the FreeSurfer recon-all pipeline, including "ribbon.mgz" and "aparc.a2009s+aseg.mgz", and local gyrification index outputs (Madan & Kensinger, 2016; Madan & Kensinger, 2017a; Madan & Kensinger, 2017b; http://cmadan.github.io/calcFD/). To assess FD, a select neuroanatomical structure on an MRI image is overlayed with a grid of boxes of a particular size (e.g., 2mm). The number of boxes that contain either the border (i.e., surface-only) or filled space within (i.e., filled volume) the structure of interest (e.g., hippocampus) are counted. The box size can be then increased, either on a fixed grid (box-counting method) or on a sliding grid scale (dilation method), and the boxes containing the structure are counted again. Fractal dimensionality is then quantified as the negative double logarithmically transformed change in the number of cubes containing the structure over change in cube size ($FD = -\frac{\Delta log2(Count)}{\Delta log2(Size)}$). Put another way, FD represents the steepness of the gradient by which increasing cube size reduces the number of cubes required to fully capture a structure. This measure captures complexity as more complex structures show greater reductions in the number of cubes required to capture them as cube size grows, owing to the larger number of cubes needed at high resolutions to capture higher complexity (See Figure 3). Accordingly, a higher value for FD represents increased structural complexity, whereas a lower FD value represents

decreased structural complexity. The current study used the filled volume method with 1mm, 2mm, 4mm, 8mm, and 16mm cubes and the dilation algorithm. These options were selected as filled volume has been shown to yield improved measurement of age-related differences (Esteban et al., 2009) and the dilation algorithm has been shown to yield superior measurements compared to the box-counting method (Madan & Kensinger, 2016; Madan & Kensinger, 2017b). FD was calculated for all cortical regions included in the Desikan–Killiany–Tourville (DKT; Klein & Tourville, 2012) atlas apart from the banks of the superior temporal sulcus, the corpus callosum, and the frontal and temporal poles, resulting in 31 cortical regions per hemisphere (Klein & Tourville, 2012). The FD of seven subcortical structures was also assessed bilaterally based on segmentation using the conventional FreeSurfer subcortical segmentation protocol (Fischl et al., 2002). Finally, the lobe-wise GM FD and the FD of four ventricular structures (i.e., third, fourth, inferior, and inferior lateral) was assessed using standard FreeSurfer segmentation labels.

Figure 2. 3

Visual representation of high and low cortical fractal dimensionality



Note. FD = fractal dimensionality

2.4.6 Quality Control

Neuroanatomical measurements for hippocampal and amygdala subregions, SM, and cortical, subcortical, lobe-wise, and ventricular FD from all participants were examined for outliers using a threshold of Z = 3.99. There were 6 outliers amongst hippocampal subfield data, none in amygdala nuclei data, 2 in SM data, and 10 in cortical FD data. Given the small amount of outlying data, outlying data was kept to maintain natural biological variability. All raw T1-weighted images were also visually inspected for quality of definition, banding, ringing, motion artifacts and anatomical abnormalities using previous established criteria (HCP Protocol Standard Operating Procedures, 2017).

2.5 Data Analytic Strategy

2.5.1 Between-Groups Analysis

To investigate differences in novel neuroanatomical measures (i.e., hippocampal and amygdalar subfield, SM, and FD) between AUD and control participants, multivariate analyses of covariance were performed using age, sex, income, years of education, and estimated total intracranial volume as covariates. To reduce the number of measures included and likelihood of type I error, an omnibus strategy was employed for subfield analysis. Accordingly, for the hippocampus, bilateral volumes for whole hippocampal head, body, and tail were first tested for between-group differences. If significant between-group differences were present, the contained subfields were then tested (see Appendix D for included nuclei). For the amygdala, the whole left and whole right amygdala were first examined for significant between group volumetric differences; if present, the contained nuclei of the amygdala (i.e., basal nucleus) were then tested for

between-group differences. An omnibus strategy was not employed for SM and FD measures as they span the entire cortical surface and as they are particularly novel in the context of AUD.

Intercorrelations between regions that demonstrated significant between group differences within each novel measure were assessed using bivariate correlations. As one AUD participant was missing data for income, this participant was excluded from this portion of analysis. All analyses were conducted using SPSS (version 28) and with a statistical threshold of $p < .05$.

2.5.2 AUD Symptom Correlational Analysis

Associations between AUD symptoms and neuroanatomical indicators that differed significantly between AUD and control participants were tested using partial correlations (two-tailed). Associations were tested between AUD severity, as assessed by the number of DSM-5 symptoms endorsed on the DART or SCID, and between each of the 11 individual DSM-5 symptoms. As with the between-group analysis, age, sex, income, years of education, and average estimated total intracranial volume were included as covariates.

2.5.3 Diagnostic Accuracy

To assess the potential for novel indicators to be used as biomarkers for AUD, the diagnostic accuracy of neuroanatomical measures that displayed significant between-group differences was tested using receiver operating characteristic (ROC) analysis. Area under the curve (AUC) was then used to assess the capability of each measure to discriminate between AUD and control participants. An AUC of 0.5 indicates no

discriminatory capability, .70-.80 indicates acceptable discriminatory capability, .80–.90 indicates excellent diagnostic accuracy, and .9–1.0 indicates outstanding discrimination (Hosmer & Lemeshow, 1991).

2.5.4 Supplemental Reliability Analysis

Given the novelty of the neuroanatomical measures in the current study, a supplementary analysis was completed to assess the reliability of each measure (i.e., hippocampal and amygdala subregions, SM, and FD). This was completed using test-retest data from the human connectome project (HCP), a large study that aimed to map the human connectome in healthy young adults. Full descriptions of the methods, including eligibility and exclusion criteria and MR image acquisition, are available elsewhere (Van Essen et al., 2012; Glasser et al., 2013). In total, 1113 individuals underwent MRI sessions which included whole brain T1-weighted anatomical scans. Of these, 45 participants later went through the same scanning protocol after an average of 140 days (sd = 67.1 days). To parallel methods in the current study, unprocessed T1-weighted images from both scanning sessions were ran through the standard FreeSurfer recon-all pipeline (version 6.0) and hippocampal subfields and nuclei of the amygdala were estimated using an additional FreeSurfer module (version 7.1). Also paralleling the current methods, SM and FD measures were generated using MATLAB.

To assess the reliability of the novel measures, intraclass correlation coefficients (ICC) were generated between test-retest data. ICC values below 0.40 indicate poor retest reliability, values between .40-.59 indicate fair retest reliability, values between .60 –.74

indicate good reliability, and values between .75–1.00 indicate excellent reliability (Cicchetti, 1994).

CHAPTER 3: HIPPOCAMPAL SUBFIELDS

3.1 Results

3.1.1 Between-Group Analysis

The first step of the between-group analysis tested the bilateral whole hippocampal head, body, and tail as omnibus tests and revealed the right whole hippocampal head to be the only subregion exhibiting significant differences between AUD and control participants (see Table 3.1). Consequently, subfields of the right hippocampal head were tested for between-group differences, revealing significantly lower right presubiculum, subiculum, and molecular layer head volumes in AUD participants relative to controls (see Table 3.2, and Figure 3.1).

Table 3. 1

Between-group differences for hippocampal subregions employed as an omnibus test

Subregion	**Mean CTRL V[a]**	**Mean AUD V[b]**	**F**	***p*-value**	**n^2_p**
L Whole Hippocampus Head	1775.75	1751.23	0.88	.349	.006
L Whole Hippocampus Body	1221.83	1207.07	0.72	.398	.005
L Whole Hippocampus Tail	586.42	573.80	1.13	.290	.008
R Whole Hippocampal Head	**1861.53**	**1795.38**	**5.20**	**.024**	**.034**
R Whole Hippocampal Body	1215.23	1183.30	2.39	.124	.016
R Whole Hippocampal Tail	570.24	571.90	0.02	.893	1.23E-04

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. V = volume. AUD = Alcohol use disorder. CTRL = control. $n^a = 79$. $n^b = 76$. Bold indicates subregions that had significantly different volume between groups.

Table 3. 2

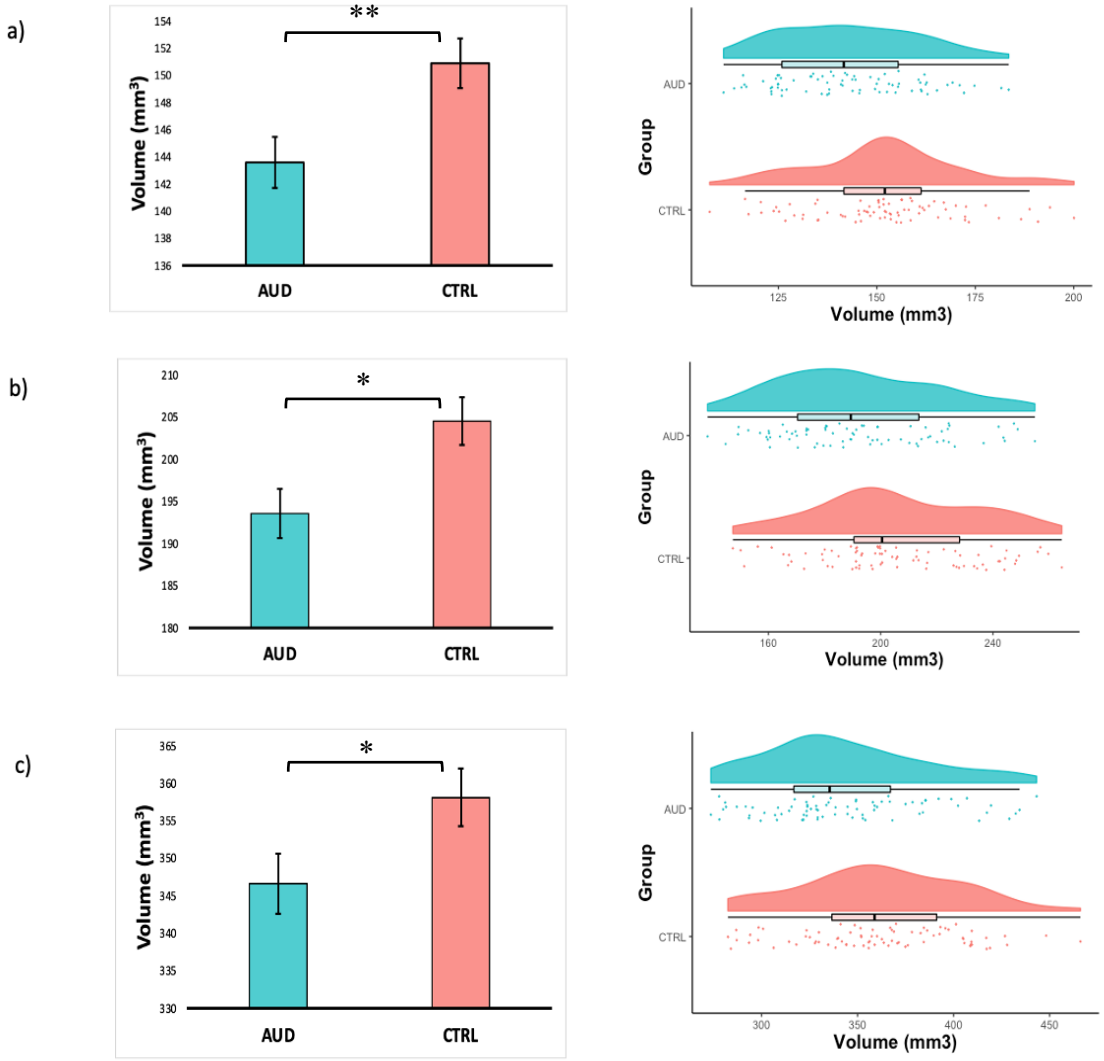
Between-group volume differences within subfields of the whole right hippocampal head

Hippocampal Subfield	**Mean CTRL V[a]**	**Mean AUD V[b]**	**F**	***p*-value**	**n^2_p**
R Parasubiculum	67.79	65.30	1.69	.196	0.011
R Presubiculum Head	**150.91**	**143.60**	**7.27**	**.008**	**0.047**
R Subiculum Head	**204.53**	**193.61**	**6.75**	**.010**	**0.044**
R CA1 Head	567.30	548.93	3.54	.062	0.023
R CA3 Head	138.25	133.67	2.00	.159	0.013
R CA4 Head	137.48	133.90	1.84	.177	0.012
R GC-ML-DG-head	167.17	162.76	1.85	.175	0.012
R Molecular Layer Head	**358.15**	**346.63**	**4.05**	**.046**	**0.027**
R HATA	69.97	66.99	3.11	.080	0.021

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. V = volume. AUD = alcohol use disorder. CTRL = control. $n^a = 79$. $n^b = 76$. Bold indicates subfields that had significantly different volume between groups.

Figure 3. 1

Mean volume of right hippocampal head subfields that differed significantly between AUD and control participants and associated rain cloud plots (a = right presubiculum head, b = right subiculum head, c = right molecular layer head)



Note. AUD = Alcohol use disorder. CTRL = controls.

*$p < .05$, **$p < .01$, *** $p < .001$

3.1.2 AUD Diagnostic Criteria Analysis

Partial correlations between right hippocampal head subfield volumes that differed significantly between AUD and control participants and individual and total number of AUD diagnostic criteria are displayed in Table 3.2. While there were no significant associations between the right presubiculum volume and total number of endorsed AUD diagnostic criteria, significant negative associations were present with substantial time devoted, alcohol cravings, and social problems (See Appendix B for full list of AUD symptoms). Similarly, the right subiculum head was not significantly associated with total number of AUD symptoms; however, there was a significant negative association with social problems. Finally, the right molecular layer head volume was also not significantly associated with total number of endorsed criteria but did display significant negative associations with inability to cut down and social problems. In the context of level of association with externalization behaviour, all three subfields were significantly associated with social problems which are theoretically highly associated with externalizing behaviour (see Table 1.1)

Table 3. 3

Partial correlations between right hippocampal head subfields with significant between-group volumetric differences and total and individual AUD diagnostic criteria

AUD Diagnostic Criteria	R Presubiculum Head V	R Subiculum Head V	R Molecular Layer Head V
Experience of Withdrawal	-.14	-.13	-.11
Development of Tolerance	-.06	-.04	-.03
Alcohol Cravings	**-.16***	-.15	-.14
Activities Given Up	-.14	-.13	-.11
Physical/ Psychological Problems	-.15	-.14	-.15
Substantial Time Devoted	**-.17***	-.13	-.11
Unable to Quit/ Cut Down	-.14	-.12	**-.18***
Larger/ Longer Drinking	-.13	-.14	-.07
Obligation Interference	-.12	-.11	-.11
Social Problems	**-.17***	**-.20***	**-.21***
Hazardous Use	-.06	.05	.04
Total Endorsed Criteria	-.16	-.15	-.15

Note. Individual diagnostic criteria are listed on a scale from lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. R = right. AUD = alcohol use disorder. V = volume. Bold denotes significant associations.

*$p < .05$, **$p < .01$, *** $p < .001$

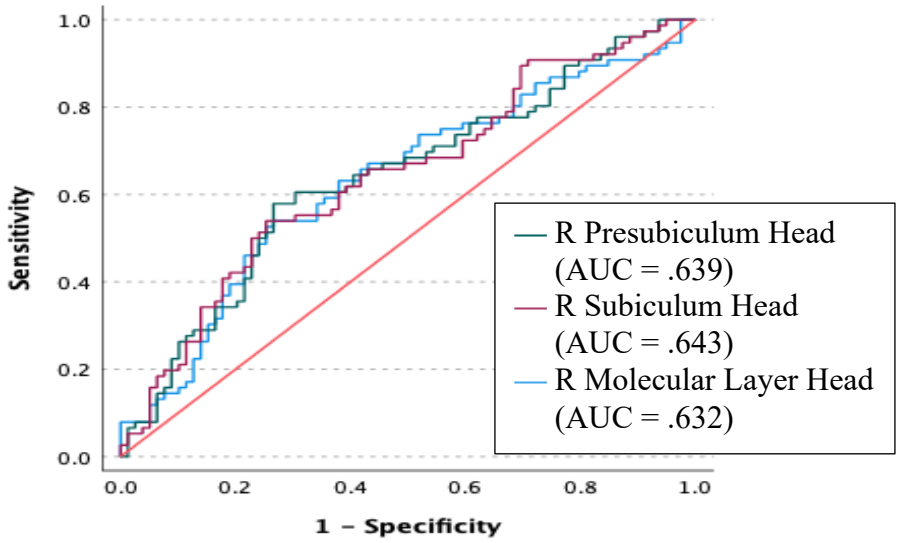
3.1.3 Diagnostic Accuracy

The classification results are given in Table 3.2. All three subfields of the right hippocampus head that differed significantly between AUD and control participants, classified AUD participants significantly ($p \leq .003$; see Table 3) better than chance. Of

the three subfields, the right subiculum head revealed the strongest diagnostic capability, with and area under the ROC curve of .643. This suggests that the volume of the right subiculum head would have an accuracy of 64.3% for differentiating individuals with and without AUD.

Figure 3. 2

Area under the receiver operating characteristics curve for right hippocampal head subfields that differed significantly between AUD and control participants



Note. AUC = area under the curve. R = right. L = left.

Table 3. 4

ROC curves for discriminating AUD participants from healthy controls using the volumes of right hippocampal head subfields that were significantly lower in AUD participants

Subfield	AUC	SE	*p* value	95% CI [LL - UL]
R Presubiculum Head	.639	0.045	.002	0.552 - 0.727
R Subiculum Head	.643	0.044	.001	0.556 - 0.730
R Molecular Layer Head	.632	0.045	.003	0.544 - 0.721

Note. AUC = area under the curve. SE = standard error. CI = confidence interval. LL = lower limit. UL = upper limit. ROC = receiver operating characteristic.

3.1.4 Reliability Analysis

Across left hippocampal subfields, ICCs ranged from .86 in the left hippocampal fissure to .97 in the left subiculum head. Similarly, across right hippocampal subfields, ICCs ranged from .83 in the hippocampal fissure to .97 in the CA1 head. Overall, results indicated excellent reliability of the automatic hippocampal segmentation algorithms, with a median ICC of .93. A complete table of ICCs can be found in Appendix D.

3.2 Interim Discussion

3.2.1 Between-Group Analysis

To further knowledge on novel structural neurocorrelates of AUD, the current study examined differences in hippocampal subfield volume between individuals with AUD and healthy controls. AUD participants exhibited significantly reduced volume in the right presubiculum, subiculum, and molecular layer head relative to controls. The right presubiculum head was the hippocampal subfield that differed the greatest between AUD and control participants. Anatomically, the presubiculum is included in the

parahippocampal area, and lies at the inferior portion of the hippocampus proper between the subiculum and the parasubiculum (Ding, 2013; see Appendix D). Functionally, animal models have pointed to important roles in episodic memory and visuospatial integration processing and integration (Honda et al., 2010; Malkova & Mortimer, 2003). Related to the current findings, volumetric reductions in the left presubiculum of individuals with DSM-4 diagnosed alcohol dependence were also discovered by Lee et al. (2016), indicating that, within the hippocampus, the presubiculum may be particularly vulnerable to alcohol related damage. Apart from addictions research, the volume of the presubiculum, along with the subiculum, has been shown to be a unique predictor of mild cognitive impairment in advance to AD (Murray et al., 2018; Carlesimo et al., 2015). Taken together, these findings highlight the presubiculum as a site of preliminary damage within the hippocampus and as a subfield with substantial potential as a biomarker.

The finding of reduced right subiculum head volume as a neurocorrelate of AUD is supported by previous research by Zahr et al. (2019), Lee et al. (2016), and Sawyer et al. (2020) who identified reduced bilateral subiculum volume in AUD, problematic drinkers, and individuals with a history of AUD, respectively. The influence of the subiculum is diverse given that it is situated at the most inferior portion of the hippocampal proper and is positioned between the CA1 and various cortices including the entorhinal cortex, as well as various subcortical structures (O'Mara, 2005). Along with the CA1, the subiculum serves as an output of the hippocampus and output from this region has been shown to play a functional role in autobiographical memory retrieval (Sawyer et al., 2020; Bartsch et al., 2011). Relatedly, select projections travelling through

the subiculum to the nuclei of the medial diencephalon and have exhibited key roles human memory and spatial learning in rodents (Aggleton & Christiansen, 2015). The subiculum also possesses connections independent of the hippocampus, such as the thalamosubiculum, which are believed to play a role in the solution of memory problems (Aggleton & Christiansen, 2015). Consequently, reduced subiculum volume within the hippocampus may be responsible for the memory deficits that are prevalent with heavy alcohol use including working memory, which relates to an individual's propensity to select immediate rewards over delayed rewards (Le Berre et al., 2017; Bickel et al., 2011). Taken together, these associated deficits may contribute to individuals with AUD having heightened discounting of future negative rewards potentially due to an impaired ability to recall past consequences. In turn, individuals with AUD are more likely to make the irrational choices to indulge in drinking behaviour despite possible negative consequences (i.e., loss of employment, negative health outcomes, etc.). Apart from memory, the subiculum also plays roles in stress response through inhibition of hippocampal mediation of the hypothalamo-pituitary-adrenocortical (HPA) axis (Herman et al., 1995). Overall, the current finding implicates the subiculum, and in particular, the right subiculum head, as a promising biomarker and treatment target.

The right molecular layer head differed the least between subfields, just passing the significant threshold. In the context of AUD, reduced right molecular layer head volume is a novel finding to our knowledge; however, research has shown that early exposure to trauma is related to reduced molecular layer head volume (Phillips et al.,

2021). Given the relationship between trauma and AUD, further exploration of this relationship may be valuable.

3.2.2 AUD Diagnostic Criteria Analysis

Given that the volumes of the right presubiculum, subiculum, and molecular layer head subfields were not significantly associated with number of AUD symptoms, the presence of significant associations with individual symptoms may offer insights into the functional antecedents or repercussions of the hippocampal subfield dysmorphology. For instance, local reductions in a select hippocampal subfield may be tied to a specific mental process that has facilitated the development of AUD, such as externalizing behaviour. Volumes of the right presubiculum, subiculum, and molecular layer head were all significantly associated with social problems which is reflective of an AUD subtype rooted in externalizing behaviour. Apart from social problems, volumetric alterations in the presubiculum were also rooted in cravings and substantial time devotion, which although less theoretically linked to externalizing behaviour, may still be an outcome of and AUD subtype driven by externalizing behaviour. Finally, volumetric deficits in the right molecular layer head appear were also related to an inability to self-regulate alcohol consumption, which is not a symptom overtly tied to externalizing behaviour. Overall, these significantly altered hippocampal subfields do not exemplify a strong neuroanatomical indicator of an AUD subtype rooted in externalizing behaviour, however, given their significant bivariate associations with social problems, it cannot be ruled out.

3.2.3 Diagnostic Accuracy Analysis

When considering diagnostic accuracy of these hippocampal subfields, neither of the three subfields reached the threshold for an acceptable level of AUD classification (i.e., AUC > .70); however, all three subfields performed were classified AUD significantly better than chance. Further research with a larger sample size or use of a composite subfield measure may reveal higher classification accuracy.

3.2.4 Reliability Analysis

Results of the reliability analysis indicated automatic segmentation of hippocampal subfields and subfield volume to have excellent (i.e., ICC = .80–.90) and outstanding (i.e., ICC: .9-1.0) reliability over time. Of note, the subfield measure with the lowest reliability in both hemispheres was the hippocampal fissure. There did not appear to be any trends in size and reliability which has been previously posited, given that the smaller regions (i.e., HATA) had equally high ICCs.

CHAPTER 4: NUCLEI OF THE AMYGDALA

4.1 Results

4.1.1 Between-Group Analysis

Between-group volumetric differences of the whole bilateral amygdala are presented in Table 4.1. The first step of the between-group analysis tested the whole bilateral amygdala volumes as an omnibus test, revealing that significant volumetric differences were localized within the left amygdala. Consequently, further analysis on the left amygdalar subfields was completed, revealing that AUD participants exhibited significantly reduced volumes in the left lateral, accessory basal, and cortical nuclei, and left corticoamygdaloid transition area volume relative to controls. Between-group differences in left amygdala nuclei volumes are visible in Table 4.2 and visual representation of significantly altered nuclei are visible in Figure 4.1.

Table 4. 1

Between-group differences for bilateral whole amygdala volume employed as an omnibus test

Hemisphere	Mean CTRL V[a]	Mean AUD V[b]	*F*	*p* value	n^2_p
L Whole Amygdala	**1720.06**	**1657.71**	**7.16**	**.008**	**0.046**
R Whole Amygdala	1811.02	1770.85	2.49	.117	0.017

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold indicates hemispheres that had significant between-group volume differences. V = volume. AUD = Alcohol use disorder. CTRL = control. $n^a = 79$. $n^b = 76$.

Table 4. 2

Between-group differences for nuclei volumes within the left amygdala

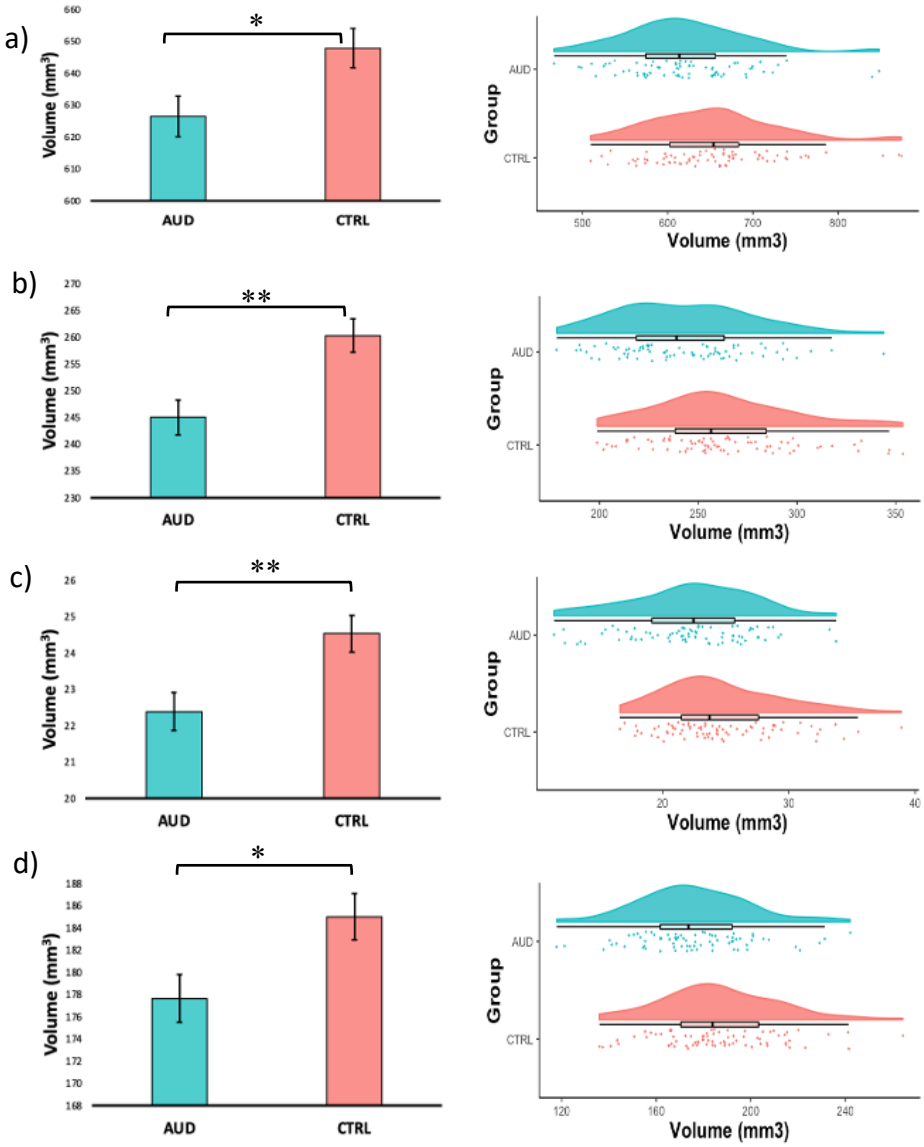
Nuclei	Mean CTRL V[a]	Mean AUD V[b]	*F*	*p*-value	n^2_p
L Lateral Nucleus	**647.76**	**626.4**	**5.42**	**.021**	**.036**
L Basal Nucleus	436.72	424.85	3.76	.054	.025
L Accessory Basal Nucleus	**260.29**	**245.05**	**10.64**	**.001**	**.067**
L Anterior-Amygdaloid-Area	52.41	51.51	0.62	.433	.004
L Central Nucleus	42.08	39.61	3.45	.065	.023
L Medial Nucleus	20.64	19.67	0.70	.404	.005
L Cortical Nucleus	**24.53**	**22.39**	**8.22**	**.005**	**.053**
L Corticoamygdaloid-Transition-Area	**184.99**	**177.63**	**5.67**	**.019**	**.037**
L Paralaminar Nucleus	50.63	50.60	0.002	.960	1.50E-05

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold indicates nuclei that had significant between-group volume differences. Volume measured in mm^3. V = volume. AUD = alcohol use disorder. CTRL = control.

$n^a = 79$. $n^b = 76$.

Figure 4.1

Mean volumes the left amygdalar nuclei that significantly differed between AUD and control participants and associated rain cloud plots (a = left lateral nucleus, b = left accessory basal nucleus, c = left cortical nucleus, d = left corticoamygdaloid-transition area)



Note. AUD = alcohol use disorder. CTRL = control.

*$p < .05$. **$p < .01$. ***$p < .001$.

4.1.2 AUD Diagnostic Criteria Analysis

Partial correlations between the four nuclei of the amygdala wherein volume differed significantly between AUD and control participants and total and individual AUD diagnostic criteria are visible in Table 4.3. Apart from the left lateral nucleus, all nuclei volumes possessed significant negative bivariate associations with total endorsed AUD diagnostic criteria, alcohol cravings, and inability to quit or cut down. Significant negative associations were present between all nuclei and the endorsement of social problems. Alternatively, no nuclei displayed significant associations with the development of tolerance, hazardous use, or larger and longer drinking.

Table 4. 3

Partial correlations between left amygdala subfields wherein volume differed significantly between AUDs and CTRLs and total and individual AUD diagnostic criteria

AUD Diagnostic Criteria	L Lateral Nucleus V	L Accessory Basal Nucleus V	L Cortical Nucleus V	L Corticoamygdaloid-Transition-Area V
Experience of Withdrawal	-.07	**-.21***	**-.20***	-.14
Development of Tolerance	.06	-.03	-.07	-.02
Alcohol Cravings	-.12	**-.18***	**-.17***	**-.18***
Activities Given Up	-.10	-.20	**-.20***	-.09
Physical/ Psychological Problems	**-.18***	**-.22****	**-.17***	-.14
Substantial Time Devotion	-.13	**-.18***	**-.20***	-.11
Unable to Quit/Cut Down	-.12	**-.25****	**-.27****	**-0.17***
Larger/Longer Drinking	-.10	-.13	-.13	-.08
Obligation Interference	-.15	**-.18***	**-.18***	-.16
Social Problems	**-.17***	**-.23****	**-.20***	**-.20***
Hazardous Use	-.14	.02	.03	-.02
Total Endorsed Criteria	-.14	**-.22****	**-.20***	**-.17***

Note. Diagnostic criteria are listed on a scale of lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold denotes significant associations. V = volume. AUD = alcohol use disorder. CTRL = control.

*$p < .05$. **$p < .01$. ***$p < .001$.

4.1.3 Diagnostic Accuracy

The classification results are given in Table 4. All left amygdalar nuclei wherein the volume differed significantly between AUD and control participants classified AUD

participants significantly ($p \leq .012$; see Table 4.4) better than chance. The left accessory basal nucleus was the best classifier, followed by the left lateral nucleus, left corticoamygdaloid-transition area, and the left cortical nucleus, respectively. The left lateral nucleus volume had an AUC of .651, indicating that this measure would discriminate between AUD and healthy individuals with an accuracy of 65.1%.

Table 4. 4

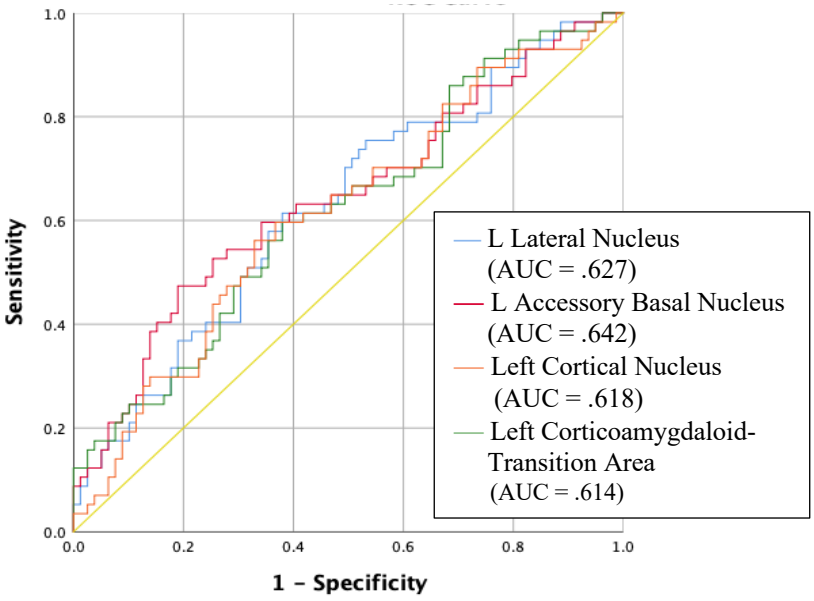
ROC curves for discriminating AUD participants from healthy controls using the volumes of left amygdalar nuclei that were significantly lower in AUD participants

Subfield	**AUC**	**SE**	***p*-value**	**95% CI [LL - UL]**
L Lateral Nucleus	.64	0.05	.004	0.548 - 0.723
L Accessory Basal Nucleus	.65	0.04	.001	0.565 - 0.737
L Cortical Nucleus	.62	0.05	.012	0.529 - 0.705
L Corticoamygdaloid-Transition Area	.63	0.05	.007	0.537 – 0.713

Note. AUC = area under the curve. SE = standard error. CI = confidence interval. LL = lower limit. UL = upper limit. ROC = receiver operating characteristic. L = left. R = right.

Figure 4.3

Area under the receiver operating characteristics curve for nuclei of the left amygdala that differed significantly between AUD and control participants



Note. AUC = area under the curve. L = left.

4.1.4 Reliability Analysis

Across nuclei of the left amygdala, ICCs range from .71 in the left medial nucleus to .96 in the left lateral nucleus, with a median ICC of .90. Similarly, across the nuclei of the right amygdala, ICCs ranged from .74 in the right central nucleus to .98 in the left lateral nucleus, with a median ICC of .91.

4.2 Interim Discussion

4.2.1 Between-Group Analysis

Results of the current study support previous findings that purport a significant association between AUD and reduced amygdala volume. However, unlike Hill et al.

(2001), AUD participants in the current study displayed significant reductions in the left whole amygdala rather than the right whole amygdala. Regarding individual nuclei of the left amygdala, the left accessory basal nucleus exhibited the largest effect size, followed by the left cortical nucleus, the left corticoamygdaloid transition area, and the left lateral nucleus, respectively. In all cases, AUD participants had significantly lower nuclei volume than controls. While reduced volume of the left cortical nucleus and corticoamygdaloid area appears to be a novel finding in relation to AUD, reduced volume of the lateral and accessory basal nuclei is not. Grace et al. (2021) found that the basolateral amygdala (BLA), which includes the accessory basal, lateral, and basal nuclei, was reduced in alcohol dependent males. The BLA has been shown to modulate stress and fear facilitated conditioning (Rodriguez Manzanares et al., 2005). This can be seen in animal models, wherein mice strains with lower BLA volume show increased fear response (Yang et al., 2008). Although, the relationship between AUD and stress is not fully understood, heightened stress reactivity has been linked to increased alcohol consumption (Clay & Parker, 2018; Seo et al., 2016), and anxiety disorders typically precede AUDs (Allan et al., 2002). Given these associations, it is not surprising that coping and negative emotion relief have been proposed as a distinct pathway to AUD (Nikolova et al., 2016). In addition, more recent evidence has implicated the BLA as playing a key role in the integration of reward incentive value, history, and cost (Wassum & Izquierdo, 2015). Taken together, it is conceivable that volume deficits in the lateral and accessory basal nuclei may provide a neurobiological basis for AUDs rooted in stress reactivity and maladaptive reward processing.

This novel finding of volumetric reductions in select nuclei within the left amygdala in AUD participants is also of particular interest given that research has implicated genetic roles in amygdala volume reduction (Zou et al., 2018; Benegal., 2006). These findings lay groundwork for future research to investigate whether these target subfields precede AUD and to shed light on their potential unique roles in stress reactivity, reward processing, and family history.

4.2.2 AUD Diagnostic Criteria Analysis

AUD diagnostic criteria analysis revealed the left accessory basal and cortical nuclei to possess the highest number of significant bivariate relationships with diagnostic criteria theoretically linked to externalizing behaviour and high severity AUD (McDowell et al., 2019; Saha et al., 2006; Proudfoot et al., 2006). In all cases, decreased volume was associated with increased endorsement of the criteria. Despite showing less relation to symptoms theoretically linked to externalizing behaviour, the left lateral nucleus and left corticoamygdaloid transition area volume were also significantly associated with social problems, which is also indicative of an AUD subtype rooted in externalizing behaviour. This is noteworthy given that reductions in bilateral amygdala volume have been linked to increased externalizing behaviour (Benegal et al., 2006).

When considering the roles that the lateral and accessory basal nuclei play in fear conditioning (Rodriguez Manzanares et al., 2005), the observed relationship between the volume of these nuclei and physical and psychological problems is also noteworthy. Research has shown that anxiety can be exacerbated with alcohol use (Kushner et al., 2000). Accordingly, this association may be indicative of the reduced volumes leading to

anxiety problems. These findings warrant further exploration into the relationship between the volume of these specific nuclei, distinct diagnostic criteria, and potential modulating factors such as anxiety disorders and family history.

4.2.3 Diagnostic Accuracy Analysis

Results of the diagnostic accuracy analysis revealed moderate potential for the left lateral, accessory basal, and cortical nuclei and the left corticoamygdaloid transition area to be used as diagnostic tools. The left accessory basal nucleus displayed the highest diagnostic potential, classifying AUD with an accuracy of 65%. While AUCs were not high enough to be considered an affective diagnostic tool, confidence intervals suggest that higher rates may be possible with a largest sample size. Further, that some nuclei classified with greater accuracy suggests that consideration of individual subfields, rather than whole amygdala measures, may be useful for classification of AUD.

4.2.4 Reliability Analysis

Reliability analysis using HCP test-retest data indicated overall excellent reliability for both the right and left nuclei. Interestingly, in both hemispheres, the lowest ICCs were yielded for the central and medial nucleus. When considering the average volumes of the amygdala nuclei, the mean volume for the left accessory basal nucleus in the sample was 430.54mm^3, whereas the mean volumes of the left central and medial nuclei were 40.83mm^3 and 20.11 mm^3, respectively. It is possible that reliability rates were slightly lower for the central and median nuclei due to their small size.

CHAPTER 5: Sulcal Morphology

5.1 Results

5.1.1 Between-Group Analysis

Results of the sulcal morphology analysis revealed significantly increased width of the left occipito-temporal, right occipital and lunate, and right marginal part of the cingulate sulci and increased depth of the right post-central sulci in AUD participants relative to controls (see Table 5.1). The largest difference in SM between AUD and control participants was found in the width of the right middle occipital and lunate sulcus, followed by the width of the right marginal part of the cingulate sulcus, the depth of the right post-central sulcus, and width of the left occipito-temporal sulcus, respectively. Of note, select sulcal width and depth measurements for certain participants were not able to be reliably estimated using the toolbox (see Madan, 2019 for further explanation). Consequently, participants with missing width or depth values for certain sulci were excluded from between-group analysis of the respective sulcal indicator category (i.e. sulcal width). Visual representations of between-group differences in SM means are visible in Figure 5.1 and SM measures that were significantly affected in AUD participants are depicted in Figure 5.2.

Table 5. 1

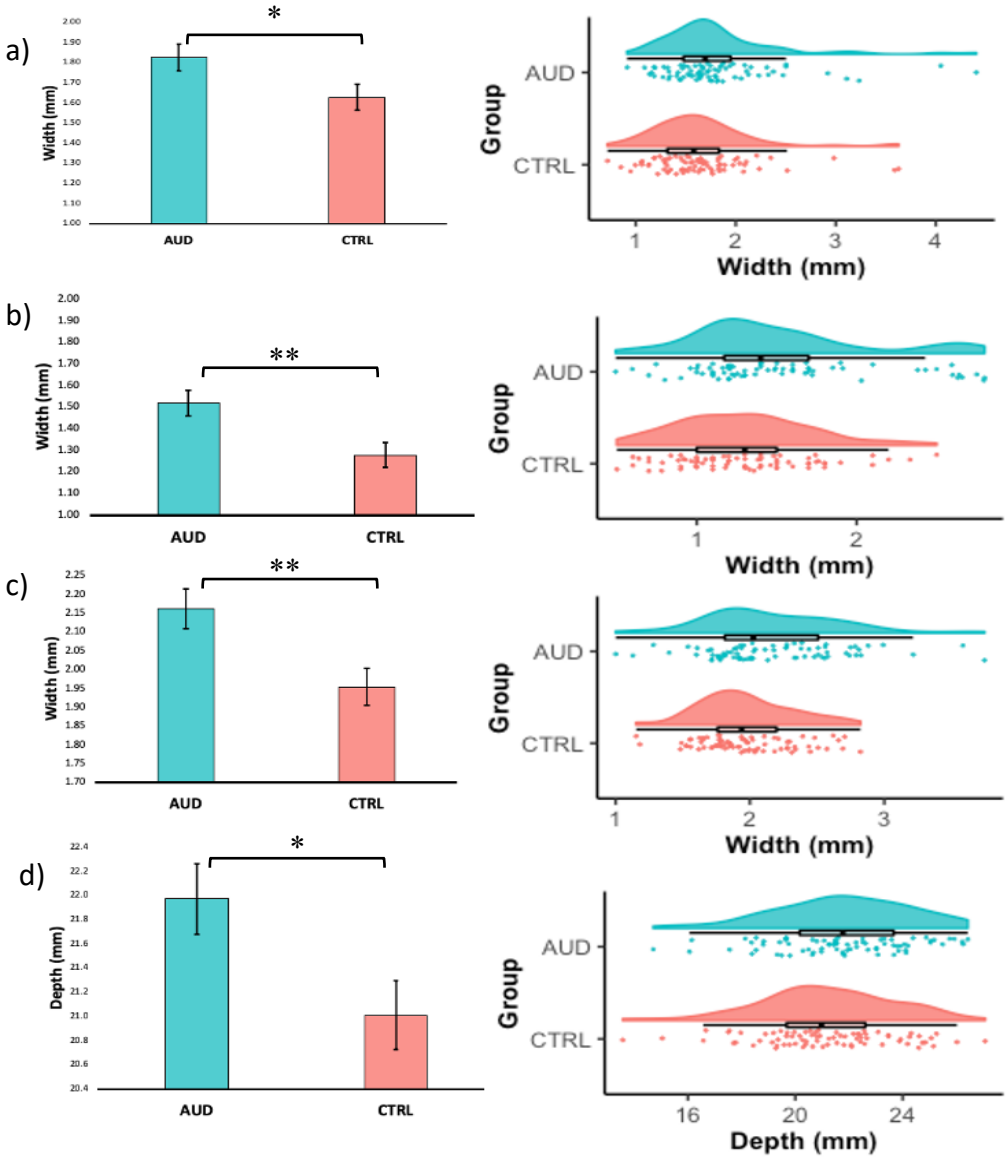
Between-group differences that were present in sulcal width and depth measures

SM region	Mean CTRL W/D	Mean AUD W/D	F	*p*-value	n^2_p
Sulcal Width					
L Occipito-temporal	1.62[a]	1.83[b]	4.30	.040	.030
R Middle Occipital and Lunate	1.28[c]	1.52[d]	7.97	.005	.054
R Marginal Part of the Cingulate	1.95[c]	2.16[d]	7.64	.006	.052
Sulcal Depth					
R Post-Central	21.01[e]	21.97[f]	5.23	.024	.035

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold indicates measures that had significantly between-group volume differences. Width and depth are measured in mm. $n^a = 73$. $n^b = 72$. $n^c = 70$. $n^d = 76$. $n^e = 75$. $n^f = 78$.W = width. D = depth. AUD = alcohol use disorder. CTRL = control. R = right. L = left. SM = sulcal morphology.

Figure 5.1

Mean sulcal width and depth that differed significantly between AUD and control participants and associated rain cloud plots (a = left occipito-temporal width, b = right middle occipital and lunate width, c = right marginal part of the cingulate width, d = right post-central depth)

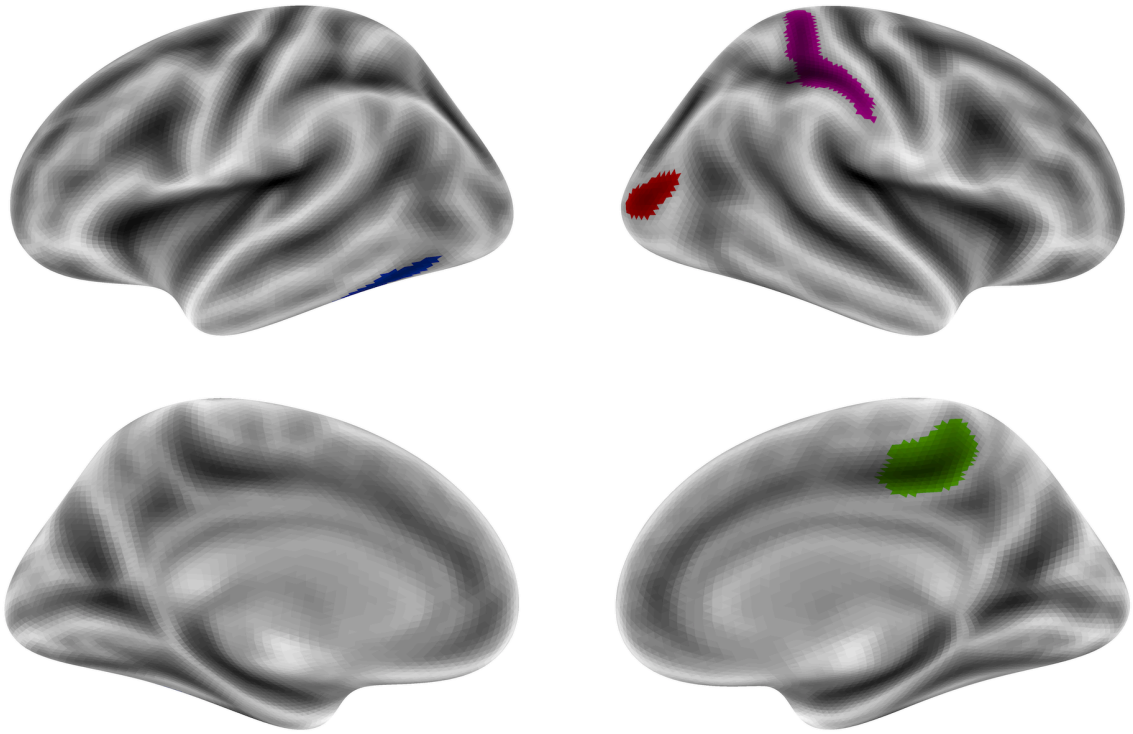


Note. AUD = alcohol use disorder. CTRL = control.

*$p < .05$. **$p < .01$. ***$p < .001$.

Figure 5.2

Visual representation of sulcal regions wherein SM differed significantly between AUD and control participants (blue = left occipital-temporal width, red = right middle occipital and lunate width, green = right marginal part of the cingulate width, purple = right post-central depth)



5.1.2 AUD Diagnostic Criteria Analysis

Partial correlations between the four SM measures that differed significantly between AUD and control participants and total and individual AUD symptoms are visible in Table 5.2. Apart from the width of the left occipito-temporal sulcus, all SM measures were significantly associated with total number of endorsed diagnostic criteria, or AUD severity. The width of the right marginal part of the cingulate was the most

abundantly associated with AUD diagnostic criteria, displaying significant associations with all criteria but obligation interference. The depth of the right post-central sulcus was significantly related to the experience of withdrawal, development of tolerance, physical and psychological problems, and inability to quit or cut down. When interpreting significant post-central depth associations by degree of externalizing behaviour (McDowell et al., 2020; see Table 1.1), significant correlations were more weighted in neuroadaptation than externalization. Contrarily, the width of the right middle occipital and lunate sulcus was significantly associated with inability to quit or cut down and hazardous use; two criteria are more linked to externalizing behaviour. Finally, the width of the left occipito-temporal sulcus was mainly associated with cravings, physical and psychological problems, and substantial time devotion; three diagnostic criteria that are not highly tied to externalizing behaviour.

Table 5. 2

Partial correlations between total and individual AUD diagnostic criteria and sulcal width and depth measures that displayed significant between-group differences

AUD Diagnostic Criteria	L Occipito-Temporal W	R Middle Occipital and Lunate W	R Marginal Part of the Cingulate W	R Post-Central D
Experience of Withdrawal	.17	.15	**.26****	**.23****
Development of Tolerance	.11	.14	**.28*****	**.20***
Alcohol Cravings	**.19***	.15	**.24****	.14
Activities Given Up	.08	.15	**.27****	.16
Physical/ Psychological Problems	**.18***	.09	**.22****	**.18***
Substantial Time Devotion	**.17***	.09	**.18***	.07
Unable to Quit/ Cut Down	.08	**.19***	**.19***	**.21***
Larger/ Longer Drinking	.05	.12	**.26****	**.24****
Obligation Interference	.16	.11	.16	.08
Social Problems	.03	.08	**.29*****	.12
Hazardous Use	-.02	**.21***	**.21***	.04
Total Endorsed Criteria	.13	**.19***	**.31*****	**.20***

Note. Individual diagnostic criteria are listed on a scale from lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold denotes significant associations. Width and depth are measured in mm. V = volume. AUD = alcohol use disorder. CTRL = control. W = width. D = depth. R = right. L = left. *$p < .05$. **$p < .01$. ***$p < .001$.

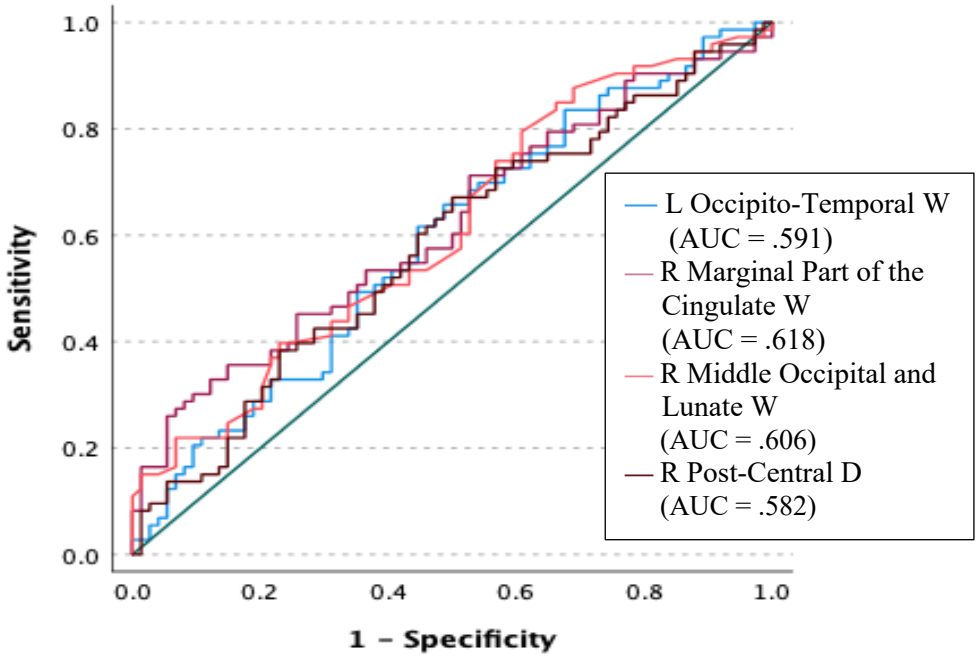
5.1.3 Diagnostic Reliability Analysis

ROC curves for SM regions that differed significantly in width or depth between AUD and control participants are visible in Figure 5.2. The right marginal part of the cingulate width displayed the highest classification accuracy with an AUC of .62,

indicating an ability to discriminate between AUD and controls with 62% accuracy. The right middle occipital and lunate and left occipito-temporal widths had the next highest classification accuracies with AUCS of . 61 and .60, indicating discriminatory accuracies of 61% and 60%, respectively. Finally, the right post-central depth had an AUC of .59 indicating a discriminatory accuracy of 59%. Aside from width of the left middle occipital and lunate and depth of the right post-central sulci, all explored SM measures were able to discriminate between AUDs and controls significantly better than chance (see Table 3).

Figure 5.2

Area under the receiver operating characteristics curve for sulcal morphology measures that differed significantly between AUD and control participants



Note. AUC = area under the curve. AUD = alcohol use disorder. R = right. L = left.

Table 5. 3

ROC curves for discriminating AUD participants from healthy controls using the volumes of left amygdalar nuclei that were significantly lower in AUD participants

Subfield	AUC	SE	*p* value	95% CI [LL - UL]
L Occipito-temporal W	.60	0.05	.057	0.50 - 0.68
R Marginal Part of the Cingulate W	.62	0.05	.013	0.53 - 0.71
R Middle Occipital and Lunate W	.61	0.05	.021	0.52 - 0.70
R Post Central D	.58	0.05	.088	0.49 – 0.67

Note. AUC = area under the curve. SE = standard error. CI = confidence interval. LL = lower limit. UL = upper limit. ROC = receiver operating characteristic. W = width. D = depth. L = left. R = right.

5.1.4 Reliability Analysis

Across sulcal width measures, ICCs ranged from .71 in the left middle occipital and lunate sulcus to .98 in the left post central sulcus, with a median ICC of .81. ICCs for sulcal width measures ranged from .88 for the depth of the right central sulcus to .98 for the right parieto-occipital sulcus. Sulcal depth measures demonstrated slightly higher reliability than sulcal width, with a median ICC of .96.

5.2 Interim Discussion

5.2.1 Between-Group Analysis

In accordance with the aims of the study, novel SM measures were explored in the context of AUD. Results revealed significantly increased width and depth of select sulci in AUD participants relative to controls. The SM measure that appeared to be the most affected by AUD was the width of the right middle occipital and lunate sulcus, followed by width of the right marginal part of the cingulate sulcus, the depth of the right post-

central sulcus, and the width of the occipito-temporal sulcus, respectively. While knowledge on the functional roles of these sulci is limited, results are likely indicative of cortical atrophy. As a novel measures, research on SM in relation to neurological conditions is rare, however, all four sulcal measurements have been found to be negatively correlated with age (Madan, 2019). Relatedly, neurotoxic damage due to drinking is often referred to as accelerated aging (Spencer & Hutchison, 1999). While there is no existing research on regional alterations in SM in relation to AUD, Wang et al. (2016) found sulci to have more pronounced cortical thickness reductions than gyri in individuals with DSM-4 alcohol dependence relative to controls. They also found that with 2 weeks of sobriety, sulcal recover exceeded that of gyral recovery; indicating that the cytoarchitecture of sulci appears to be particularly vulnerable to alcohol-related damage.

5.2.2 AUD Diagnostic Criteria Analysis

When considering associations between SM measurements that differed significantly in AUD participants and AUD diagnostic criteria, the width of the right marginal part of the cingulate sulcus displayed substantially more significant associations with criteria than the other sulcal measures. In particular, moderate associations were present with all criteria except for obligation interference. These associations suggest that the widening of this sulcus is associated with externalizing behaviour and that this finding may be prevalent in individuals with an externalizing subtype of AUD, such as type B or II (Cloninger et al., 1996). However, given the significant relationships are not limited to a particular subset of criteria, widening of

the right marginal part of the sulcus may instead be due to the neurotoxic effects of ethanol and may prove effective as a biomarker for heavy alcohol use as a whole. The other SM measures do not display such a clear trend, however, widening on the right middle occipital and lunate sulcus was also associated with hazardous use which is robustly associated with externalizing behaviour and related AUD subtypes. Given widening of the right middle occipital and lunate sulcus only showed significant associations with two individual criteria. Consequently, this finding may represent morphological alternations that are limited to AUD subtypes rooted in externalizing behaviour. Alternatively, the width of the left occipito-temporal sulcus only displayed relationships with criteria considered to be less severe (Saha et al., 2006; Proudfoot et al., 2006). Taken together, these results highlight that SM measures are differentially related to AUD diagnostic criteria and implicate the widths of the left occipito-temporal, right middle occipital and lunate, and right marginal part of the cingulate sulci, and depth of the post-central sulcus as candidate biomarkers for AUD. Further, the diversity in these relationships supports the theory of distinct structural differences underlying specific pathways for AUD development.

5.2.3 Diagnostic Accuracy Analysis

Classification analysis revealed differential classification ability of the sulcal measures that differed significantly between AUDs and controls. While the right marginal part of the cingulate and middle occipital and lunate sulci could classify AUD with over 60% accuracy, the width of the occipito-temporal sulcus and the depth of the post-central sulcus displayed 59% and 58% accuracy, respectively. This indicates that, of the novel

measures explored in the current study, SM measures have slightly less diagnostic potential. However, given the sample size is relatively small for classification analysis, further research is required to solidify this finding.

5.2.4 Reliability Analysis

Results of the HCP reliability analysis revealed excellent reliability for sulcal depth measures and good reliability for sulcal width measurements. The measurement reliability of this toolbox has also been validated using previous test-retest data in a separate dataset (Madan, 2019). Results of the previous analysis revealed ICCs which indicated good to excellent reliability for sulcal depth (ICC = .85 - .98) and good reliability for sulcal width (ICC = .76 - .86) measurements, except for the widths of the bilateral parieto-occipital, occipito-temporal, and middle occipital and lunate sulci which indicated moderate reliability (ICC = .61 - .69).

CHAPTER 6: FRACTAL DIMENSIONALITY

6.1 Results

6.1.1 Between-Group Analysis

Cortical parcels wherein FD differed significantly between AUD and control participants are visible in Table 6.1 and Figure 6.1. Of the 31 cortical parcels in the left hemisphere that were tested for between-group FD differences, the medial OFC and pars opercularis were the only two that exhibited significant differences. Both cortical parcels displayed lower FD in AUD participants relative to healthy controls. Of the 31 cortical parcels tested in the right hemisphere, the right lateral occipital cortex was the only parcel that displayed significant between-group differences. Unlike the left cortical parcels, FD was significantly higher in AUDs relative to controls. Subcortical structures wherein FD differed significantly between AUD and control participants are visible in Table 6.2, Figure 6.2, and Figure 6.4. Of the eight subcortical structures in the left hemisphere that were tested for FD differences, the left caudate and left thalamus exhibited significantly lower FD in AUDs relative to controls. Of the eight subcortical structures tested in the right hemisphere, the right pallidum and putamen exhibited significantly lower FD in AUDs relative to controls. Ventricles that displayed significant FD differences between AUDs and controls are visible in Table 6.3 and Figure 6.3. Of the four ventricles tested for between-group differences, the third ventricle and inferior lateral ventricle displayed significantly increased FD relative to controls. No significant group differences were observed for lobe-wise FD.

Table 6. 1

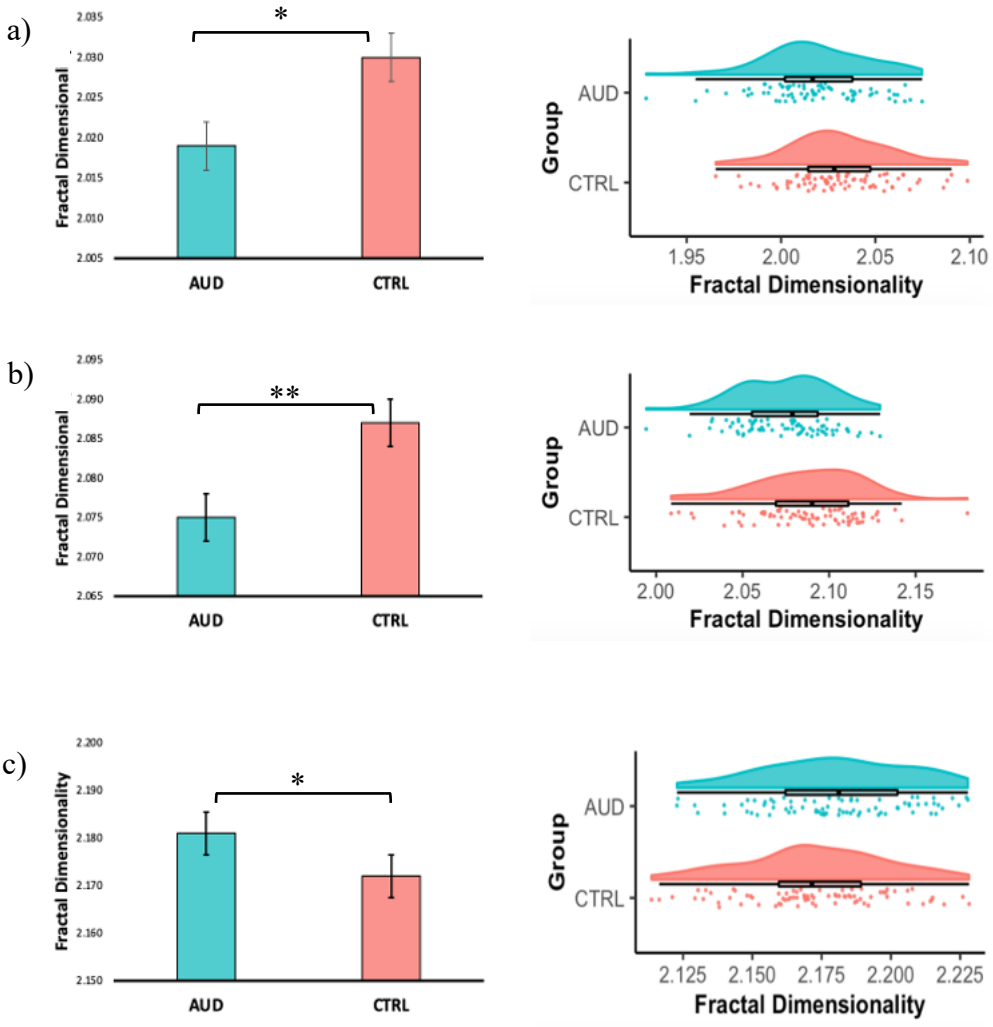
FD measures which differed significantly between AUD and control participants

Region	Mean CTRL FD[a]	Mean AUD FD[b]	F	*p*-value	n^2_p
Cortical					
L Medial OFC	2.03	2.02	5.40	.022	.035
L Pars Opercularis	2.09	2.08	8.51	.004	.055
R Lateral Occipital	2.17	2.18	4.87	.029	.032
Subcortical					
L Caudate	2.30	2.29	14.40	2.16E-04	.089
L Thalamus	2.30	2.30	6.40	.012	.042
R Pallidum	1.98	1.97	5.10	.025	.034
R Putamen	2.09	2.09	7.91	.006	.051
Ventricles					
Third	1.76	1.80	6.76	.010	.044
Inferior Lateral	1.36	1.41	5.86	.017	.038

Note. Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. FD = fractal dimensionality. AUD = alcohol use disorder. CTRL = control. OFC = orbitofrontal cortex. $n^a = 79$. $n^b = 76$.

Figure 6.1

Cortical parcels wherein mean FD differed significantly between AUD and control participants and associated rain cloud plots (a =left medial OFC, b = left pars opercularis, c = right lateral occipital cortex)

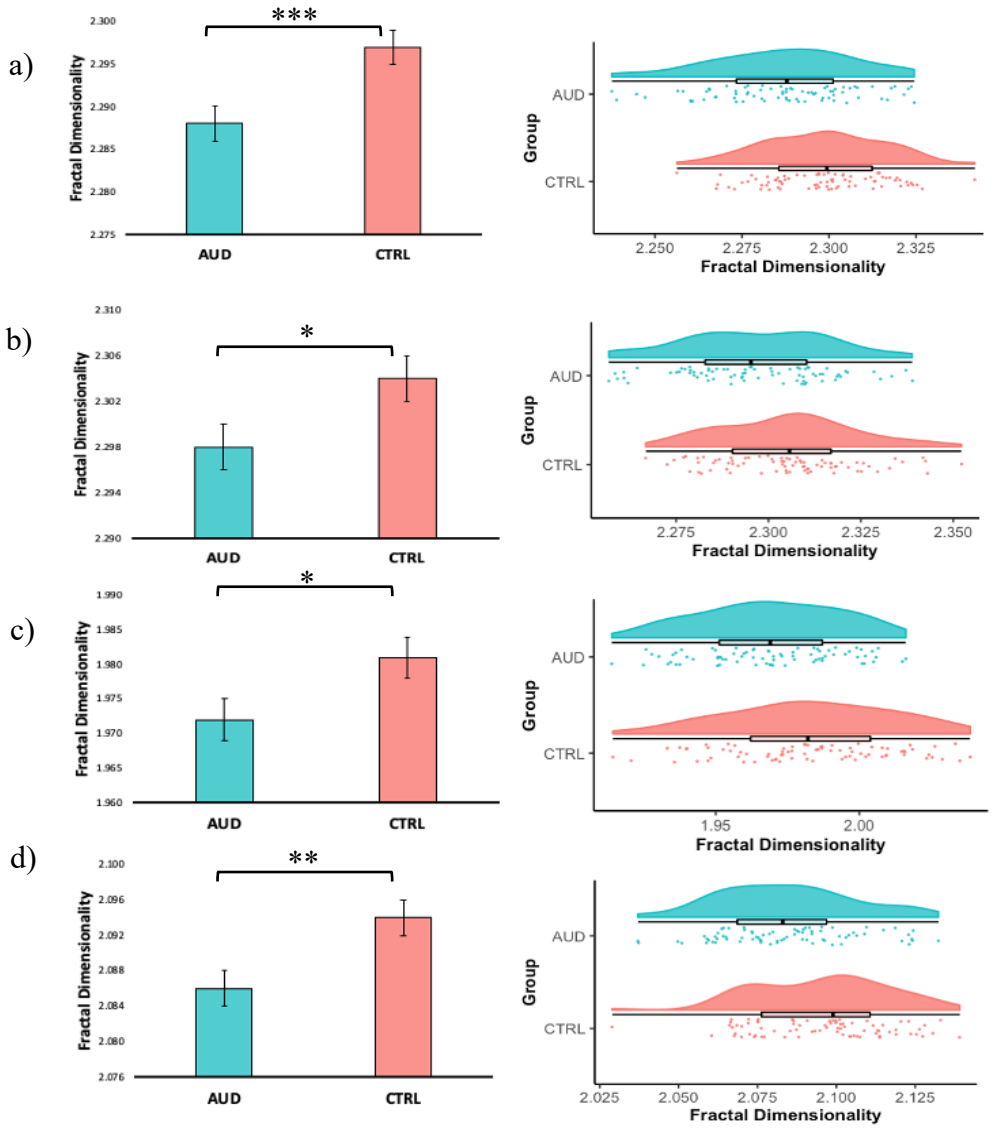


Note. AUD = Alcohol use disorder. CTRL = controls.

*$p < .05$, **$p < .01$, *** $p < .001$

Figure 6.2

Subcortical regions wherein mean FD differed significantly between AUD and control participants and associated rain cloud plots (a =left caudate, b = left thalamus, c = right pallidum, d) right putamen)

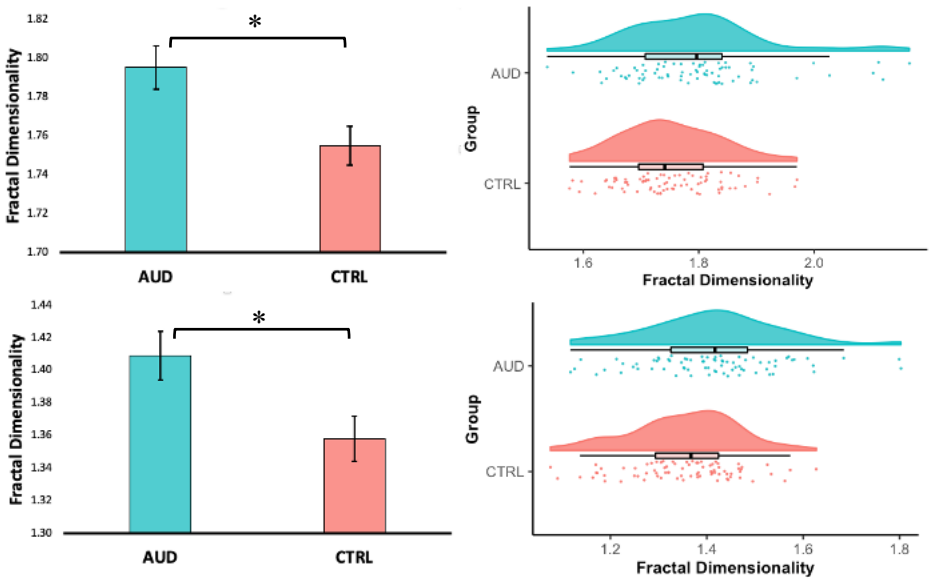


Note. AUD = Alcohol use disorder. CTRL = controls.

*$p < .05$, **$p < .01$, *** $p < .001$

Figure 6.3

Ventricles wherein mean FD differed significantly between AUD and control participants and associated rain cloud plots (a = third ventricle, b = inferior lateral ventricle)

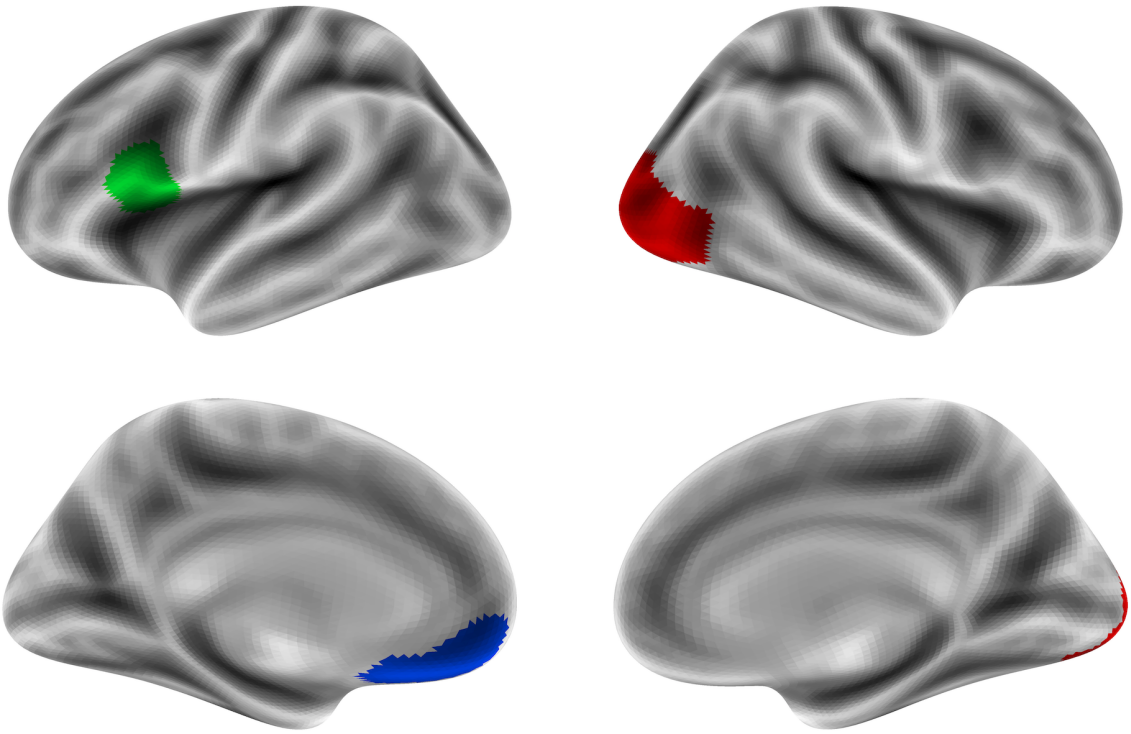


Note. AUD = Alcohol use disorder. CTRL = controls.

*$p < .05$, **$p < .01$, *** $p < .001$

Figure 6.4

Visual representation of cortical parcels wherein FD differed significantly between AUD and control participants (green = left pars opercularis, red = lateral occipital cortex, blue = medial OFC)



6.1.2 AUD Diagnostic Criteria Analysis

Partial correlations between cortical parcels wherein FD differed significantly between AUD and control participants are visible in Table 2. All three parcels displayed significant bivariate associations with total AUD diagnostic criteria endorsed. Apart from total endorsed diagnostic criteria, the left medial OFC also displayed significant associations with inability to quit or cut down alcohol use and obligation interference, two symptoms higher rated moderately high externalizing behaviour scale. The left pars opercularis displayed significant bivariate associations with all diagnostic criteria except for development of tolerance and hazardous use. Finally, the right lateral occipital cortex displayed significant bivariate associations with development of tolerance and inability to quit or cut down drinking, symptoms which are not particularly linked with externalizing behaviour.

Bivariate associations with subcortical structures wherein FD differed significantly are visible in Table 2. Similar to cortical results, the FD of all subcortical structures displayed significant associations with total endorsed diagnostic criteria. The left caudate displayed moderate significant bivariate associations with all diagnostic criteria apart from hazardous use. Similarly, FD of the right putamen was moderately associated with all diagnostic criteria aside from hazardous use and activities given up. Although the left thalamus and right pallidum were both differentially associated with individual diagnostic criteria, neither displayed overt trends with criteria particularly linked to externalizing behaviour. Regarding ventricles that exhibited significant between-group differences in FD, the third and inferior lateral ventricles were both

significantly associated with total endorsed criteria. Both ventricular FDs also displayed abundant significant bivariate relationships with individual AUD criteria. The third ventricle was significantly related to all criteria except for hazardous situations, relationship problems, and obligation interference, the three criteria most readily linked to externalizing behaviour. The inferior lateral ventricle which was significantly associated with all criteria aside from hazardous use and development of tolerance.

Table 6. 2

Partial correlations cortical parcels wherein FD differed significantly between AUDs and CTRLs and individual and total endorsed AUD criteria

AUD Diagnostic Criteria	L Medial OFC	L Pars Opercularis	R Lateral Occipital
Experience of Withdrawal	-.15	**-.19***	.14
Development of Tolerance	-.06	-.09	**.16***
Alcohol Cravings	-.13	**-.25****	.08
Activities Given Up	-.13	**-.28*****	.14
Physical/Psychological Problems	-.08	**-.18***	.14
Substantial Time Devotion	-.15	**-.19***	.14
Unable to Quit/Cut Down	**-.22****	**-.21***	**.17***
Larger/Longer Drinking	-.06	**-.18***	.14
Obligation Interference	**-.18***	**-.19***	.06
Social Problems	-.16	**-.21***	.11
Hazardous Use	.05	-.14	.12
Total Endorsed AUD Criteria	**-.17***	**-.24****	**.18***

Note. Diagnostic criteria are listed on a scale of lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold denotes significant associations. V = volume. AUD = alcohol use disorder. CTRL = control. R = right. L = left.

*$p < .05$. **$p < .01$. ***$p < .001$.

Table 6. 3

Partial correlations between subcortical structures wherein FD differed significantly between AUDs and CTRLs and individual and total endorsed AUD criteria

AUD Diagnostic Criteria	L Caudate	L Thalamus	R Pallidum	R Putamen
Experience of Withdrawal	**-.20***	-.15	**-.18***	**-.18***
Development of Tolerance	**-.22****	-.15	-.01	**-.17***
Alcohol Cravings	**-.27****	**-.18***	-.10	**-.24****
Activities Given Up	**-.20***	-.11	**-.20***	-.15
Physical/Psychological Problems	**-.17***	-.12	**-.22****	**-.20***
Substantial Time Devotion	**-.24****	**-.16***	**-.20***	**-.18***
Unable to Quit/Cut Down	**-.32*****	**-.22****	-.16	**-.17***
Larger/Longer Drinking	**-.24****	-.15	-.12	**-.19***
Obligation Interference	**-.23****	-.18	-.15	**-.20***
Social Problems	**-.25****	**-.17***	**-.22****	**-.21***
Hazardous Use	-.15	-.08	-.01	-.01
Total Endorsed AUD Criteria	**-.28*****	**-.19***	**-.18***	**-.22****

Note. Diagnostic criteria are listed on a scale of lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold denotes significant associations. V = volume. AUD = alcohol use disorder. CTRL = control. R = right. L = left.

*$p < .05$. **$p < .01$. ***$p < .001$.

Table 6. 4

Partial correlations between ventricles wherein FD differed significantly between AUDs and CTRLs and individual and total endorsed AUD criteria

AUD Diagnostic Criteria	Third Ventricle	Inferior Lateral Ventricle
Experience of Withdrawal	**.18***	**.22****
Development of Tolerance	**.20***	.11
Alcohol Cravings	**.23****	**.24****
Activities Given Up	**.20***	**.26****
Physical/Psychological Problems	**.22****	**.16***
Substantial Time Devotion	**.24****	**.22****
Unable to Quit/Cut Down	**.20***	**.21***
Larger/Longer Drinking	**.21***	**.18***
Obligation Interference	.15	**.19***
Social Problems	.14	**.17***
Dangerous Situations	.07	-.04
Total Endorsed AUD Criteria	**.23****	**.21***

Note. Diagnostic criteria are listed on a scale of lowest to highest association with externalization behaviour based on McDowell et al. (2019). Age, sex, estimated average intracranial volume, income, and years of education were included as covariates. Bold denotes significant associations. V = volume. AUD = alcohol use disorder. CTRL = control. R = right. L = left.

*$p < .05$. **$p < .01$. ***$p < .001$.

6.1.3 Diagnostic Reliability Analysis

Results of the diagnostic reliability analysis using FD measures are visible in Tables 6.5. All FD measures that differed significantly between AUD and controls were able to discriminate between AUD and controls significantly ($p \leq .021$) better than

chance, apart by the right lateral occipital cortex. AUCs ranged from .58 for the right lateral occipital cortex FD to .65 by the left pars opercularis and right putamen FD.

Table 6. 5

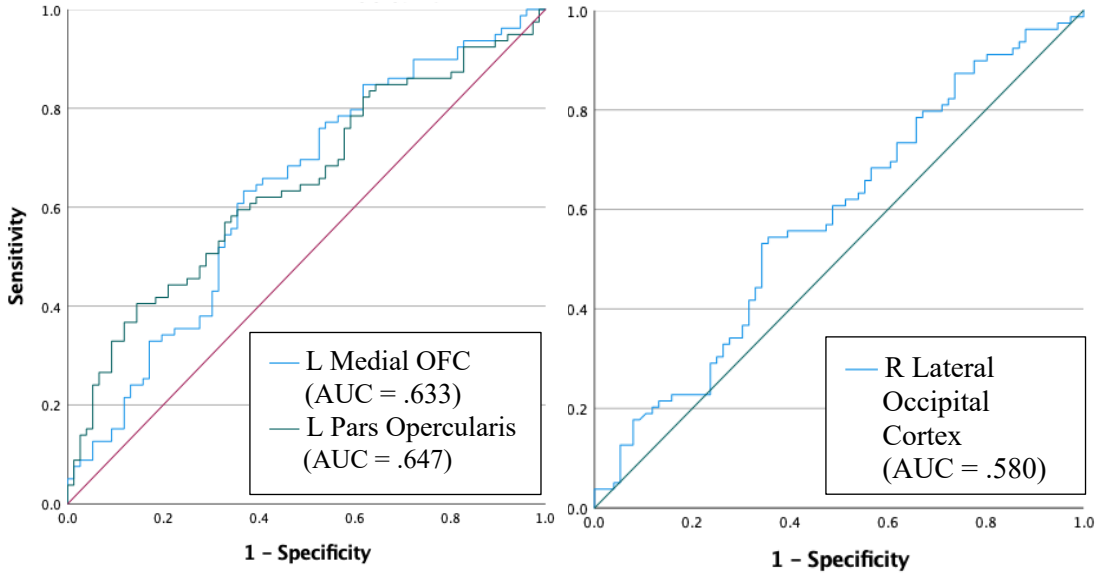
ROC curves for discriminating AUD participants from healthy controls using the volumes of left amygdalar nuclei that were significantly lower in AUD participants

FD Measure	AUC	SE	*p*-value	95% CI [LL – UL]
L Medial OFC	.63	0.05	.004	0.55 – 0.72
L Pars Opercularis	.65	0.04	.001	0.56 – 0.73
R Lateral Occipital Cortex	.58	0.05	.087	0.49 – 0.67
L Caudate	.65	0.04	.001	0.57 – 0.74
L Thalamus	.62	0.05	.013	0.53 – 0.70
R Pallidum	.64	0.04	.003	0.55 – 0.73
R Putamen	.65	0.04	.001	0.57 – 0.74
Third Ventricle	.63	0.05	.005	0.54 – 0.72
Inferior Lateral Ventricle	.61	0.05	.021	0.52 – 0.70

Note. AUC = area under the curve. SE = standard error. CI = confidence interval. LL = lower limit. UL = upper limit. ROC = receiver operating characteristic. OFC = orbitofrontal cortex.

Figure 6.5

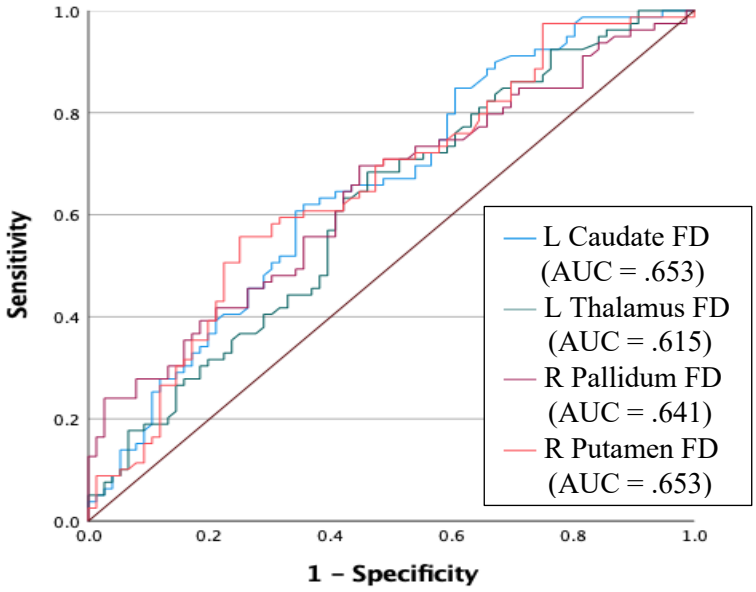
Area under the receiver operating characteristics curve for cortical parcels wherein FD differed significantly between AUD and control participants



Note. AUD = alcohol use disorder. AUC = area under the curve. OFC = orbitofrontal cortex. L = left. R = right.

Figure 6.6

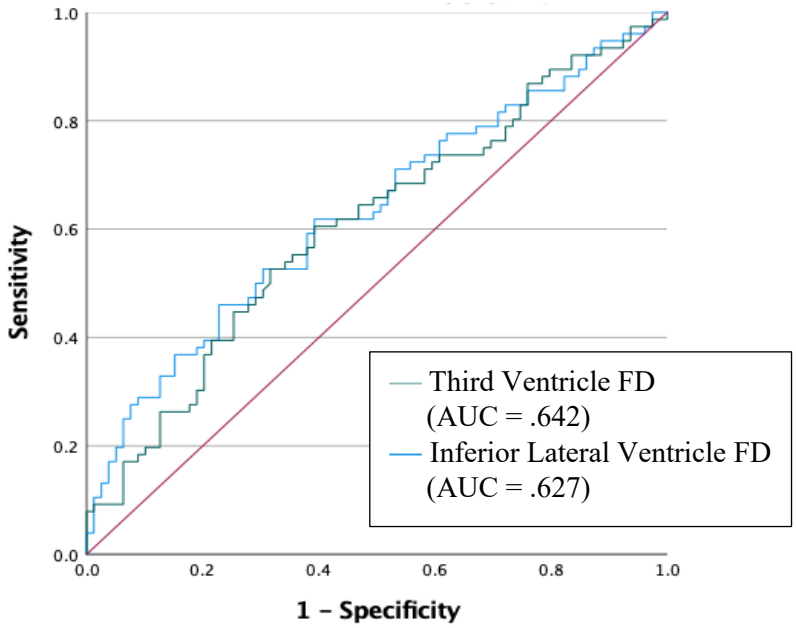
Area under the receiver operating characteristics curve for subcortical structures wherein FD differed significantly between AUD and control participants



Note. AUD = alcohol use disorder. AUC = area under the curve. OFC = orbitofrontal cortex. L = left. R = right. FD = fractal dimensionality.

Figure 6.7

Area under the receiver operating characteristics curve for ventricles wherein FD differed significantly between AUD and control participants



Note. AUD = alcohol use disorder. AUC = area under the curve. R = right. L = left. FD = fractal dimensionality.

6.1.4 Reliability Analysis

Across left cortical parcels, ICCs for FD measures ranged from .76 in the entorhinal cortex to .98 in the middle temporal gyrus, with a median ICC of .94. FD measures for right cortical parcels ranged from .65 in the parahippocampal gyrus to .98 in the middle temporal gyrus, with a median ICC of .94. Across left subcortical structures, ICCs for FD estimates ranged from .58 in the accumbens to .94 in the putamen, with a median ICC of .82. Right subcortical FD measurement ICCs ranged from .58 in the amygdala to .90 in the caudate, with a median ICC of .88. Ventricular FD measurement

ICCs ranged from .91 in the lateral ventricle to .98 in the fourth ventricle, with a median ICC of .97. Lobe-wise FD measurements in the left hemisphere ranged from .89 in the occipital lobe to .96 in the frontal lobe, and from .61 in the occipital lobe to .80 in the frontal lobe in the right hemisphere. Median ICC for lobe-wise FD was .94 and .73 in the left and right hemispheres, respectively.

6.2 Interim Discussion

6.2.1 Between-Group Analysis

The current study endeavored to identify novel biomarkers for AUD by exploring novel neuroanatomical measures, such as FD. By estimating FD on systematically parcellated cortical regions, the whole cortical surface was considered, eliminating any potential biases by limiting the investigation to a priori ROIs. While this method did result in a large number of variables (i.e., 31 cortical parcels per hemisphere), thus increasing risk of type I error, it suited the exploratory aims of the study. The cortical FD analysis revealed three cortical parcels, the left medial OFC, the left pars opercularis and right lateral occipital cortex, to possess significantly lower FD, or complexity, in AUD participants relative to controls. Interestingly, both the left medial OFC and pars opercularis displayed reduced FD (i.e., complexity), whereas the right lateral occipital cortex showed increased FD. While research employing FD measures to alcohol is nonexistent, existing research on FD and other neurological processes and conditions that relate to cognitive decline, such as Alzheimer's disease, minimal hepatic encephalopathy, and aging, have consistently found reductions in cortical FD (Chen et al., 2020; King et al., 2010; Madan, 2016). Given these findings and the small effect size ($n^2_p = 0.032$), this

finding should be interpreted with caution. The cortical region with the largest effect size was the left medial OFC. The result of reduced FD in the left medial OFC is supported by Durazzo et al. (2011), where regional volume in the left medial OFC was significantly reduced in DSM-4 diagnosed alcohol-dependent participants. Of note, in the same study, CT and cortical SA were not significantly altered in AUD participants. Taken together, these findings suggest that FD may be a more sensitive measure of alcohol-related cortical alterations than CT or SA. This finding is also significant as CT of the medial OFC has also been discovered to be reduced in offspring with a family history of alcohol misuse (Henderson et al., 2018). While further longitudinal research is required to deduce whether FD alterations reflect neurotoxic effects or may precede AUD, taken together, these studies implicate the medial OFC as a promising biomarker.

Subcortical FD analysis revealed the left caudate, left thalamus, right pallidum, and right putamen to have significantly lower FD, or complexity, in AUD participants relative to controls. The largest effect size ($n^2_p = .089$) of FD was observed in the left caudate, followed by the right putamen, left thalamus, and right pallidum, respectively. Reduced FD in the left caudate is of particular interest as caudate dysmorphology has been previously linked to alcohol misuse (Sullivan et al., 2005). Functional imaging studies have also found increased connections between the caudate and the motor cortex of individuals with AUD, which are theorized to relate to the increased habitual tendencies prevalent in the disorder (Seo et al., 2016). Reduced complexity of the left thalamus is also notable as reduced thalamus volume has been identified in offspring from multigenerational alcohol dependant families (Benegal et al. 2006), suggesting

morphological alterations may antedate AUD. Overall, findings demonstrate the utility of FD measures of subcortical structures and implicate the left caudate and thalamus and right putamen and pallidum as promising biomarkers for AUD.

Finally, ventricular FD analysis revealed increased FD, or complexity, of the third and inferior lateral ventricles in AUD participants relative to controls. This increased complexity is likely due to the enlargement of the ventricles which is a common finding in AUD individuals (Dager et al., 2015; Jernigan et al., 1991). Further, enlargement of the third ventricle has been implicated as a biproduct of alcohol-related age acceleration (Pfefferbaum et al., 2001). Research has also shown that ventricular enlargement recedes with abstinence (Zahr et al., 2016; Tomasi et al., 2021). Indeed, Tomasi et al. (2021) revealed a 6.4% and 24.7% reduction in the third ventricle and inferior lateral ventricle volume, respectively, after 3 weeks of abstinence. These findings suggest that these alterations are due to the neurotoxic effects of alcohol rather than being an alteration that precedes the disorder.

6.2.2 AUD Diagnostic Criteria Analysis

AUD diagnostic criteria revealed FD of select cortical parcels to be differentially associated with individual diagnostic criteria. Of the three parcels that has significantly altered FD in AUD participants, the left pars opercularis possessed the highest number of significant associations with AUD criteria and included four of the five criteria that are the most theoretically linked to externalizing behaviours (i.e., problems in relationships and obligation interference; see Table 1.1). These findings suggest, that of these parcels, alterations in the left pars opercularis may be more related to a subtype of AUD rooted in

externalizing behaviour (e.g., Type II [Cloninger et al., 1981] or Type B [Babor et al., 1992]). Contrarily, the left medial OFC and right lateral occipital cortex were more related to criteria which are related to neuroadaptation (i.e., development of tolerance, inability to quit/cut down). Similarly, decreased FD of the left caudate and right putamen were significantly associated with the majority of the AUD diagnostic criteria, including four of the five criteria that are more highly associated with externalizing behaviour. This finding is of particular interest as research has linked caudate activity to externalizing behaviour (Shannon et al., 2009). While the left thalamus and right pallidum had only one significant association highly linked to externalizing behaviour, both had significant associations with problems in relationships, a common symptom in proposed AUD subtypes rooted in externalizing behaviour. Reduced right thalamus volume has been previously found to be associated with increased externalizing behaviour (Benegal et al., 2006); as such, this finding warrants further exploration. Increased FD of the third and inferior lateral ventricles were associated with increased occurrence of several of the diagnostic criteria. Of the two, the inferior lateral ventricle was significantly associated more criteria linked to externalizing behaviour. While further research is required, these results implicate select alterations in cortical, subcortical, and ventricular FD as being potential markers for AUD subtypes derived from externalizing behaviour.

6.2.3 Diagnostic Reliability Analysis

Results of the diagnostic accuracy analysis indicated that, apart from the right lateral occipital cortex, all cortical, subcortical, and ventricular regions explored have potential as diagnostic tools for AUD. In particular, the left pars opercularis, left caudate,

and right putamen demonstrate an ability to classify AUDs with an accuracy of 65%. While these rates do not meet the threshold for an acceptable diagnostic tool (> 70%; Metz, 1978), further exploration with a larger sample may increase these rates.

6.2.4 Reliability Analysis

Reliability analysis using test-retest data from the HCP dataset revealed excellent reliability for cortical FD measurements in both hemispheres. However, select FD measurements did display below excellent reliability; FD measurements for the bilateral entorhinal cortices displayed good reliability and the right parahippocampal gyrus displayed moderate reliability. FD measurements for subcortical structures revealed good overall reliability; however, the FD measurements for the left accumbens, right thalamus and right amygdala displayed moderate reliability, and left caudate, left amygdala, and right accumbens displayed good reliability. While lobe-wise FD measurements in the left hemisphere revealed predominantly good and excellent reliability, FD measurements for the right frontal, parietal, lobes displayed good reliability, and occipital lobe FD displayed moderate reliability. All ventricular FD measures indicated excellent reliability.

CHAPTER 7: DISCUSSION

7.1 Summary of Findings

The primary aim of the current study was to explore novel measures, including hippocampal and amygdala subfields, SM, and FD, in relation to AUD and identify whether significant differences existed between individuals with AUD and healthy controls. Within hippocampal subfields, the volumes of the right presubiculum, subiculum, and molecular layer heads were significantly reduced in AUD participants relative to controls. Within the left amygdala, the left lateral, accessory basal, and cortical nuclei and the left corticoamygdaloid transition area had significantly lower volumes in AUD participants relative to controls. Sulcal morphology analysis revealed AUD participants to have significantly wider occipito-temporal, middle occipital and lunate, and marginal part of the cingulate sulci, and a deeper post-central sulcus. FD analysis revealed reduced cortical complexity of the left medial OFC and pars opercularis of AUD participants. In addition, increased cortical complexity of the right lateral occipital cortex in AUD participants was observed, however, due the small effect size, this finding should be interpreted with caution. When considering FD of subcortical structures, results revealed decreased complexity of the left caudate and thalamus and right pallidum and putamen. Finally, ventricular FD analysis revealed increased complexity of the third and inferior lateral ventricles. While impossible to discern given the cross-sectional nature of the study, research suggests that cortical and ventricular findings, such as reduced cortical FD and increased ventricular FD, show more recovery with abstinence and are more likely to be a consequence of the neurotoxic effects of alcohol (Durazzo et al., 2011;

Fortier et al., 2011). Contrarily, subcortical findings, including decreased hippocampal and amygdala subfield volume, and decreased FD of select subcortical structures, represent more permanent changes that are potentially more rooted in neurobiological vulnerability (Benegal et al., 2006; Dager et al., 2015; Zou et al., 2018). Overall, longitudinal research employing these novel measures is required to elucidate whether the observed changes precede or are due to AUD; however, the dysmorphology nonetheless demonstrates the potential utility of these novel measures as biomarkers for AUD.

The second aim of the study was to delineate observed between-group differences by testing associations with individual AUD diagnostic criteria. Several of the novel measures revealed significant bivariate associations with individual AUD criteria that are highly associated with externalizing. In particular, the left accessory basal and cortical nuclei volumes, width of the marginal part of the cingulate sulcus, and FD of the right caudate, and left caudate were found to be highly associated with criteria theoretically linked to externalizing behaviours.

The final aim of the study was to assess whether any of the novel measures included in the study could be used to classify AUD with high enough accuracy to demonstrate potential as a diagnostic tool. While none of the novel measures reached classification accuracies high enough to be considered an adequate diagnostic tool, the majority were able to classify AUD significantly better than chance. Results warrant further exploration with a larger sample size.

7.2 Limitations and Future Directions

There are several limitations of the current study worth noting and that may be addressed in future research. First, the study relied on automatic segmentation techniques using $1mm^3$ resolution, which has been cautioned as being potentially unreliable (Wisse et al., 2020). However, analysis using HCP test-retest data in the current study revealed ICCs that indicated excellent reliability. In addition, given manual segmentation requires approximately 50 hours per participant (Iglesias, 2015), automatic segmentation permits larger sample sizes and statistical power that would not be otherwise possible. Further, automatic segmentation eliminates the possibility of inter- and intra-rater variability (Leemput et al., 2009). Next, as AUD participants from the ICM study were in treatment, most of them were abstinent from alcohol. As it has been established that there is some degree of volume recovery with abstinence, particularly within the cortex (Durazzo et al., 2011; Fortier et al., 2011), it is possible that morphological differences may have been attenuated. However, inclusion of these participants permitted increased power and corresponding group sample sizes, which was valuable to the study. In addition, due to the cross-sectional nature of the study, it is impossible to discern whether the observed effects are due to premorbid brain alterations or neurotoxic effects of alcohol. Further longitudinal research is required to identify the origin of the observed dysmorphology. Finally, the current study lacked knowledge on whether participants were taking medications during the scan. Medications may have potential effects on MRI results; thus, this should be assessed in structural biomarker research (Hafeman et al., 2012).

7.3 Conclusion

While research on risk factors and structural correlates of AUD have been prevalent over the previous 20 years, effective biomarker and treatment strategies for AUD remain elusive. Advancements in neuroimaging, including increased MR resolution and automatic segmentation, grant the potential for novel measures to be considered in large samples; two factors which may be key to elucidating the neuroanatomical underpinnings of AUD. Further, shedding light on the neural correlates of distinct subtypes of AUD may delineate the breadth of current structural findings which are often diffuse and discrepant. Results of the current study implicate selectively decreased hippocampal and amygdalar subfield volume and alterations in sulcal morphology and fractal dimensionality as potential novel biomarkers for AUD. In addition, differential bivariate relations were revealed between select novel measures and individual AUD diagnostic criteria with some more heavily related to externalizing behaviour. Taken together, results highlight the potential of these novel measures to be employed as promising biomarkers and differentiating tools for AUD subtypes.

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Appendix A: Full Inclusion and Exclusion Criteria

Table 1

Full inclusion and exclusion criteria for NeuroAlc and ICM studies

Criteria		Both Studies	NeuroAlc Only	ICM Only
Inclusion	Both	Fluent English speaker	21-55 years old Right-handed	25-55 years old
	AUD	Current AUD Diagnosis (>3 AUD Symptoms)	3+ AUD diagnostic criteria endorsed High-risk drinking per NIAAA guidelines (>14/7 drinks per week for ♂/♀)	4+ AUD diagnostic criteria endorsed
	Control	No DSM-5 AUD diagnosis Low-risk drinking per NIAAA guidelines (≤14/7 drinks per week for ♂/♀)	Must report minimum weekly drinking	Must report minimum monthly drinking

Exclusion	History of schizophrenia-spectrum/psychotic disorders or bipolar disorders History of neurological disorders (e.g., Parkinson's disease, multiple sclerosis) History of significant brain injury (e.g., stroke, traumatic brain injury) MRI contraindications (e.g., pregnancy/ breastfeeding, metal implants, claustrophobia)	Currently in treatment for alcohol or other substance use disorder DSM-5 substance use disorder other than nicotine or cannabis	History of post-traumatic stress disorder History of neurocognitive disorder or impairment DSM-5 substance use disorder other than nicotine Attending a study session with a positive breath alcohol concentration (BrAC > 0.00g%)

Appendix B: Between Study Characteristic Comparisons

Table 1

Comparison of AUD participant characteristics in NA and ICM studies

Variable (mean [SD] / %)	NA AUD[a]	ICM AUD[b]	Mean Difference	t	p	d
Age	33.22 [10.85]	36.63 [9.65]	-6.42	-2.63	**.010**	0.11
Sex (% Female)	63.0	33.3	0.30	2.61	**.011**	0.61
Handedness (%RH)	100	96.6[c]	0.10	1.72	.089	0.41
Income (Median)	$60,000 - $75,000	$30,000 - $45,000	2.08	3.50	**8.0E-4**	0.83
Years of Education	14.74 [2.89]	14.43 [2.62][c]	0.31	0.47	.641	0.11
Race (% Eastern	84.8	86.7 [c]	0.05	0.60	.552	0.14

European)						
# AUD symptoms	6.59 [2.59]	9.03 [1.43]	-2.45	-4.73	**1.1E-5**	-1.11
Drinks/Week	23.00 [15.92]	-	-	-	-	-

Notes. [a]n = 46. [b]n = 30. [c]n = 29. **ICM drinking data for AUD participants is not included as some were in treatment. Bold indicates significant differences.*

Table 2

Comparison of AUD participant characteristics in NA and ICM studies

Variable (mean [SD] / %)	NeuroAlc CTRL[a]	ICM CTRL[b]	Mean Difference	t	p	d
Age	31.33 [9.83]	36.78 [10.18]	-5.44	-2.34	**.022**	-0.54
Sex (% Female)	56.7	61.2	-0.05	-0.40	.693	-0.09
Handedness (% RH)	100	87.8	0.08	1.30	.198	0.30
Income (Median)	$60,000 - $75,000	$60,000 - $75,000	-0.50	-0.85	.401	-0.20
Years of Education	16.03 [2.46]	17.12 [3.46]	-1.09	-1.51	.136	-0.35
Race (% Eastern European)	83.3	85.7	0.002	0.28	.778	0.07
# AUD symptoms	0 [0.0]	0.04 [0.20]	-0.04	-1.12	.268	-0.26
Drinks/week	4.81 [3.31]	5.33 [5.58]	0.51	-0.46	.649	-0.11

Notes. [a]n = 30. [b]n = 49. **ICM drinking data for AUD participants is not included as some were in treatment*

Appendix C: Diagnostics and Statistical Manual 5th Edition Criteria for Alcohol Use Disorder

(5th ed.; DSM-5; American Psychiatric Association, 2013).

A. A problematic pattern of alcohol use leading to clinically significant impairment or dis tress, as manifested by at least two of the following, occurring within a 12-month period:

1. **Larger/Longer Drinking**: Alcohol is often taken in larger amounts or over a longer period than was intended.
2. **Unable to Quit/Cut Down**: There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
3. **Substantial Time Spent:** A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
4. **Alcohol Cravings:** Craving, or a strong desire or urge to use alcohol.
5. **Obligation Interference:** Recurrent alcohol use resulting in a failure to fulfill major role obligations at work, school, or home.
6. **Social Problems:** Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
7. **Give Up Activities**: Important social, occupational, or recreational activities are given up or reduced because of alcohol use.
8. **Hazardous Use:** Recurrent alcohol use in situations in which it is physically hazardous.
9. **Physical/ Psychological Problems:** Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.
10. **Tolerance Development**: Tolerance, as defined by either of the following:
    - A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
    - A markedly diminished effect with continued use of the same amount of alcohol.
11. **Experience of Withdrawal**: Withdrawal, as manifested by either of the following:
    - The characteristic withdrawal syndrome for alcohol
    - Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms.

Appendix D: Novel Measures Included in Analysis

Table 1

Hippocampal measures analyzed in each hemisphere

Both Hemispheres (Y/N)	Subfield	Subregion
Y	Parasubiculum	Head
Y	Presubiculum Head	
Y	Subiculum Head	
Y	CA1 Head	
Y	CA3 Head	
Y	CA4 Head	
Y	GC-ML-DG-head	
Y	Molecular Layer Head	
Y	HATA	
Y	Presubiculum Body	Body
Y	Subiculum Body	
Y	CA1 Body	
Y	CA3 Body	
Y	CA4 Body	
Y	GC-ML-DG Body	
Y	Molecular Layer Body	
Y	Fimbria	
Y	Hippocampal Tail	Tail
Y	Fissure	Fissure

Note. CA = cornu ammonis. HATA = hippocampal amygdala transition area. GC-ML-DG = granule cell and molecular layer of the dentate gyrus.

Table 2

Amygdala nuclei explored per hemisphere

Both Hemispheres (Y/N)	Nuclei
Y	Lateral Nucleus
Y	Basal Nucleus
Y	Accessory Basal Nucleus
Y	Anterior-Amygdaloid-Area
Y	Central Nucleus
Y	Medial Nucleus
Y	Cortical Nucleus
Y	Corticoamygdaloid-Transition-Area
Y	Paralaminar Nucleus

Table 3

Sulcal morphology measures explored

Both Hemispheres (Y/N)	Sulcus	Sulcal Measure
Y	Central	Depth
Y	Superior Frontal	
Y	Inferior Frontal	
Y	Post-Central	
Y	Middle Occipital and Lunate	
Y	Marginal Part of the Cingulate	
Y	Parieto-Occipital	
Y	Occipito-temporal	
Y	Central	Width
Y	Superior Frontal	
Y	Inferior Frontal	
Y	Post-Central	
Y	Middle Occipital and Lunate	
Y	Marginal Part of the Cingulate	
Y	Parieto-Occipital	
Y	Occipito-temporal	

Table 4

Lobe-wise, subcortical, and ventricular fractal dimensionality measures explored

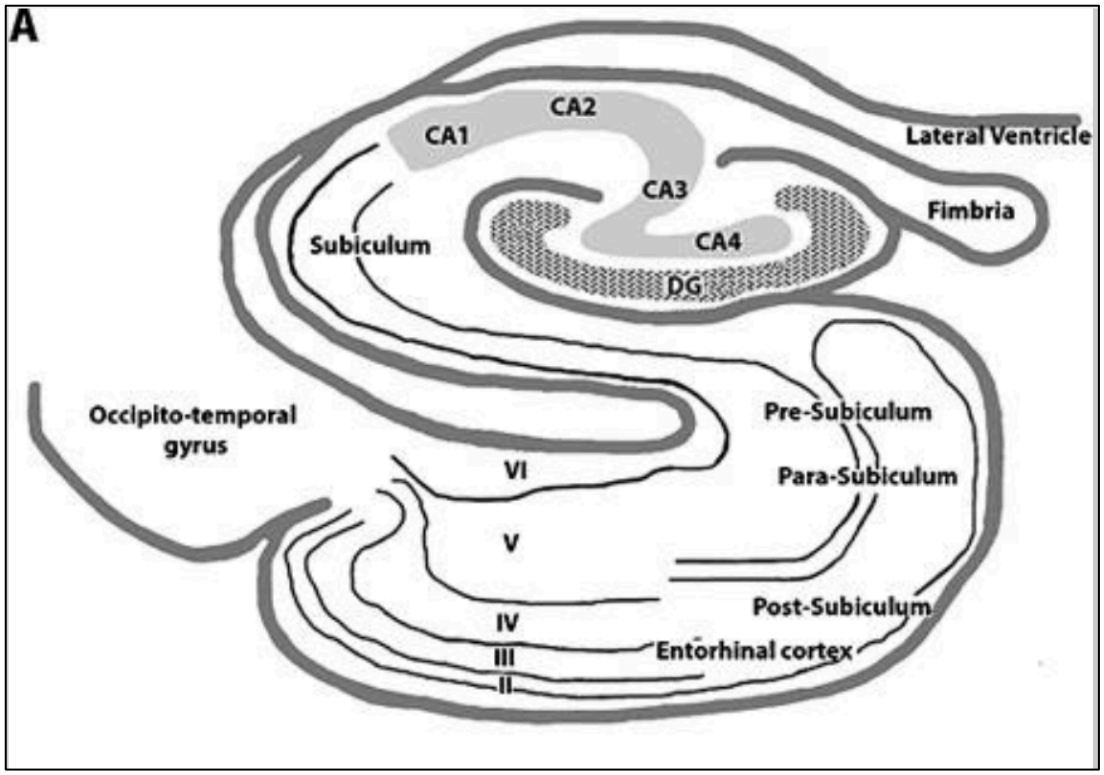
Both Hemispheres (Y/N)	Region
Y	Frontal Lobe
Y	Parietal Lobe
Y	Temporal Lobe
Y	Occipital Lobe
Y	Thalamus
Y	Caudate
Y	Putamen
Y	Pallidum
Y	Hippocampus
Y	Amygdala
Y	Accumbens
N	Lateral Ventricle
N	Inferior Lateral Ventricle
N	3rd Ventricle
N	4th Ventricle

Table 4

Cortical fractal dimensionality measures explored

Both Hemispheres (Y/N)	Region
Y	Caudal Anterior Cingulate
Y	Caudal Middle Frontal
Y	Cuneus
Y	Entorhinal
Y	Fusiform
Y	Inferior Parietal
Y	Inferior Temporal
Y	Insula
Y	Isthmus Cingulate
Y	Lateral Occipital
Y	Lateral Orbitofrontal
Y	Lingual
Y	Medial Orbitofrontal
Y	Middle Temporal
Y	Parahippocampal
Y	Paracentral
Y	Pars Opercularis
Y	Pars Orbitalis
Y	Pars Triangularis
Y	Pericalcarine
Y	Post-central
Y	Posterior Cingulate
Y	Precentral
Y	Precuneus
Y	Rostral Anterior Cingulate
Y	Rostral Middle Frontal
Y	Superior Frontal
Y	Superior Parietal
Y	Superior Temporal
Y	Supramarginal
Y	Transverse Temporal

Appendix E: Schematic representation of a transverse section of the hippocampus proper and parahippocampal regions



Note. The hippocampus can be subdivided into the dentate gyrus (DG), the hippocampus proper (cornu ammonis [CA] regions), and the subiculum. The parahippocampal region includes the presubiculum, parasubiculum, and postsubiculum. The subiculum lies between the hippocampus proper and the parahippocampal regions. Entorhinal cortex layers are depicted with roman numerals. From Stafstrom 2005.