The Impact of Alcohol Consumption on Brain Networks: Investigating Differential Effects in Bipolar Disorder

by

Fiona Martyn, B.Sc.

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School of Psychology

College of Arts, Social Sciences, & Celtic Studies

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Supervisors

Professor Gary Donohoe

Dr Dara M Cannon

Table of Contents

Chapter Four

Chapter Six

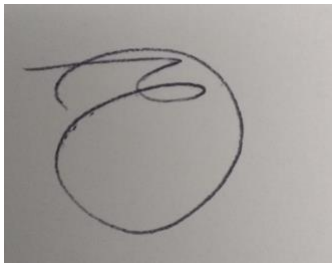
Author's Declaration

I declare that all the work presented in this thesis was carried out in accordance with the regulations of the National University of Ireland Galway. All work is original and carried out by Fiona M Martyn at the Centre for Neuroimaging & Cognition and Genomics (NICOG), Clinical Neuroimaging Laboratory, NCBES Galway Neuroscience Centre, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, H91 TK33 Galway, Ireland. This thesis has not been submitted previously for any other academic award.

The MRI and clinical data used in this thesis was gathered between 2015-2019, by Dr Pablo Najt, Dr Genevieve McPhilemy, Dr Leila Nabulsi and Fiona M Martyn.

This research was funded by the Health Research Board (HRA-POR-324) awarded to Dara M. Cannon, PhD, and the National University of Ireland, College of Arts, Social Sciences and Celtic Studies, Galway Doctoral Fellowship awarded to Fiona M Martyn.

Signed: Date: 30.03.22



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Thank you to my family: my parents Eamonn and Mary, who often told me that knowledge is never a burden, I suppose I have to admit that you're right. Cheers to my brother David for planting the seed of a life as a researcher in my head and being a co-student with me, and my brother Philip, who I was able to collaborate with during this project.

Without the support of friends over the years I would not have been able to weather the storms of moving continents, pandemics and PhD stipends. Thank you to Donncha and Tara for always falling asleep when I mentioned this project, I learned a lot about improving my science communication from you two. Thanks to the Boss Aunties: Hannah May Caspar

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To Aidan, for the clearing.

Finally, to Ruby: despite not taking your advice to work day and night so that I could finish this faster, I hope I have made you proud. Watching your curiosity for the world grow daily is the best gift I could ever wish for, I just hope you see the benefits of the metric system one day.

To the Research Participants: I gratefully acknowledge the participants of our study who gave up their time to provide us with their data, the Wellcome Trust Health Research Board, Centre for Advanced Medical Imaging (CAMI), St. James's Hospital, Dublin, and the HRB Clinical Research Facility, Galway.

Abstract

Background:

Widespread alterations to cortical regions particularly in areas related to reward processes have been described following non-dependent alcohol use and independently in bipolar disorder (BD). Additionally, alterations in the microstructural organisation of white matter are associated with non-dependent alcohol consumption and within BD. Moreover, alcohol use in the disorder impacts functional networks related to emotion, cognition, and introspection, these alterations are associated with mood lability and negative illness trajectory. Identifying the interactive and network level effects of alcohol use and diagnosis on the brain may elucidate the impact of alcohol on reward and affective circuitry and its contribution to relapse in BD. This thesis uses a range of MRI modalities and theoretical developments in network neuroscience to uncover previously unknown knowledge about the impact of alcohol on the brain and its compound effects for people with a diagnosis of BD.

Methods:

For all studies alcohol use was assessed using the Alcohol Use Disorders Identification Test, Consumption sub score (AUDIT-C). This test validly and reliably assesses alcohol consumption across a range of levels and a number of differing testing contexts.

Study One: Forty-six psychiatrically healthy and 40 BD (DSM-V-TR) participants underwent T1-weighted (MPRAGE) MRI scanning at 3T and the AUDIT-C. Cortical regions of interest were parcellated based on the Desikan-Killiany atlas (Freesurfer v.5.3.0) and included the anterior cingulate (ACC), dorsolateral prefrontal (dlPFC), and orbitofrontal

cortices (OFC), and insula. Cortical thickness was examined for an effect of alcohol use and compared between BD and control groups covarying for age, sex and diagnosis.

Study Two: Thirty-four BD-I (DSM-IV-TR) and 38 psychiatrically healthy controls underwent T1 and diffusion-weighted MRI scanning, and the AUDIT-C. Connectomes comprising 34 cortical and nine subcortical nodes bilaterally (Freesurfer v5.3) connected by fractional anisotropy-weighted edges derived from non-tensor based deterministic constrained spherical deconvolution tractography (ExploreDTI v4.8.6) underwent permutation-based topological analysis (NBS v1.2) and were examined for effects of alcohol use and diagnosis-by-alcohol use accounting for age, sex and diagnosis.

Study Three: Forty BD-I (DSM-IV-TR) and 46 psychiatrically-healthy controls underwent T1 and resting state functional MRI scanning, and the AUDIT-C. Functional images were decomposed using spatial independent component analysis, into 14 resting state networks (RSNs), which were examined for effect of alcohol use and diagnosis-by-alcohol use accounting for age, sex and diagnosis.

Results:

There was no difference in alcohol use scores between BD or control participants in all of the studies.

Study One: For all participants, alcohol use was associated with reduced cortical thickness of the left ACC (T=-2.984, p_{FDR}=0.016), left OFC (T=-2.508, p_{FDR}=0.025), and left insula (T=-2.385, p_{FDR}=0.025). For BD participants only, there was an association between alcohol use and cortical thickness in the left dlPFC (T=-2.237, p=0.032).

Study Two: Alcohol was significantly related to a subnetwork, encompassing connections between fronto-limbic, basal ganglia and temporal nodes (F_{range}=5-8.4, p=0.031). A portion of this network (18%), involving cortico-limbic and basal ganglia connections, was

differentially impacted by alcohol in the BD relative to the control group (F_{range}=5-8.8, p=0.033),

Study Three: For BD participants greater alcohol use was associated with increased connectivity of the paracingulate gyrus within a default mode network (DMN) and reduced connectivity within an executive control network (ECN) relative to controls. Independently greater alcohol use was associated with increased connectivity within an ECN, and reduced connectivity within a DMN.

Conclusions:

Taken together these papers suggest that alcohol is associated with structural alterations to specific reward related structures, is found to impact subnetwork connectivity, and that these alterations are further reflected in aberrant functional connectivity of networks subserving introspective and affective processes. Moreover, for those with a diagnosis of BD, these results suggest that pre-existing structural and functional alterations may be placed at additional vulnerability to the impact of alcohol use, which is reflected in alterations to network patterning between structures subserving affective and cognitive processes. This altered pattern of connectivity may impact on reward expectancies and emotion processing, thus influencing emotional lability within the disorder. These results indicate that clinical guidelines should reflect the impact that alcohol use may have on illness trajectory for people with a diagnosis of BD.

Publications and Science Communication Arising From This Thesis

PUBLICATIONS

Martyn, F.M, Cannon, D.M. *et al* (2021). Topological alteration is associated with non-dependent alcohol use in bipolar disorder (**Study Two**: Published in *Brain Connectivity*).

Martyn, F.M, Cannon, D.M. *et al* (2021). Alcohol Use Impacts Cortical Reward Network Structure in Bipolar Disorder (**Study One**: Submitted to *Alcohol*).

Martyn, F.M, Cannon, D.M. *et al* (2021). Alcohol use is Associated with Affective and Interoceptive Network Alterations in Bipolar Disorder (**Study Three**: Submitted to Bipolar Disorders)

ORAL PRESENTATIONS

Martyn, F.M., Cannon, D.M. *et al* (2018). Alcohol use is Associated with Reduced Anterior Cingulate Cortex Thickness in Bipolar Disorder and Healthy Participants'. Journal of Anatomy, 232, 304-355. Doi:10.111/joa.12751

Presented at Anatomists on the Edge 2017, NUI Galway.

ABSTRACTS

Martyn, F.M., Cannon, D.M. *et al* (2021). Alcohol use impacts cortical reward network structure in bipolar disorder. Biological Psychiatry 89(9):S365-S366. DOI: 10.1016/j.biopsych.2021.02.910

Online poster presentation at Society for Biological Psychiatry 76th Annual Meeting, April 2021.

Martyn, F.M., Cannon, D.M. *et al* (2020). Neuroanatomical alteration is associated with moderate alcohol use in bipolar disorder. *Biological Psychiatry, 87(9), S205.*DOI:10.1016/j.biopsych.2020.02.532

Online poster presentation at Society for Biological Psychiatry 75th Annual Meeting, April 2020.

Martyn, F.M., Cannon, D.M. *et al* (2020). Moderate alcohol use impacts connectivity of emotional and salience networks differently in bipolar disorder. European Neuropsychopharmacology 31:S71. Doi:10.1016/j.euroneuro.2019.12.095.

Poster presentation at ECNP Workshop on Neuropsychopharmacology for Early Career Scientists in Europe, 5-8 March 2020, Nice, France

Martyn, F.M., Cannon, D.M. *et al* (2019). Structural network alteration in bipolar disorder is associated with moderate alcohol use.

Poster presentation at NUI Galway, College of Medicine, Nursing, and Health Sciences Postgraduate Research Day 2019.

Martyn, F.M., Cannon, D.M. *et al* (2017). Alcohol use is Associated with Reduced Anterior Cingulate Cortex Thickness in Bipolar Disorder and Healthy Participants'. Journal of Anatomy, 232, 304-355. Doi:10.111/joa.12751

Poster presentation Neural Networks in Health and Disease, 2017, Cambridge UK.

SCIENCE COMMUNICATION

Soapbox Science, Galway, September 2021.

- I was selected to deliver a talk to a lay audience through the global Soapbox Science network based on my research addressing non-dependent alcohol use.

Galway Mayo Institute of Technology (GMIT), April 2021.

- I delivered a talk entitled*: Parties and Pathways* through the library service for students and staff of GMIT across their campuses in the West of Ireland.

Impact of Alcohol on the Brain for Non-dependent Drinkers, Hepatitis Victoria, 2021

- Podcast episode with Dr Eimear McMahon where I discuss my research on non-dependent alcohol use and the brain (Martyn & McMahon, 2021).

We Need to Talk About Ireland's Problem with Alcohol, RTE Brainstorm, 2020

- I wrote a short piece outlining the cultural issues surrounding alcohol use inherent in Irish society, and the impact on young people's alcohol consumption (Martyn, 2020).

ENCP's Got Talent, 33rd ENCP Congress, 2020

- I presented a talk entitled, *Pathways and parties: Alcohol and brain networks*, and was selected as the winner of this Early Career Researcher talent competition for presenting scientific evidence through the medium of comedy (Martyn, 2020).

Mood Atlas, NUIG, December 2019

- I was a member of the organisational team who delivered a Knowledge Exchange and Dissemination Grant (KEDS), HRB and produced a 26-minute TV ready short film which was launched in December 2019. I was filmed presenting my research for the short film. This short film is being submitted to international film festivals by the film makers: Ishka Films. Mood Atlas has won Best Medical Documentary at Sci-On Film Festival 2020, a science film festival based in Reno USA (Mullarkey, 2019).

Threesis, NUIG, November 2019

- I was selected for the reserve list for the final of the NUIG thesis competition, where I presented my PhD thesis in three slides in under three minutes.

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List of Abbreviations

Anterior cingulate cortex (ACC)

Alcohol use disorder (AUD)

Amygdala (Amy)

AUDIT-(C): Alcohol Use Disorders Identification Test (Consumption)

Bipolar disorder (BD)

Blood Oxygen Level Dependent (BOLD)

Brief Young Adult Alcohol Consequences Questionnaire (B-YAACQ)

Caudate (Caud)

CC: Clustering Coefficient

Cerebrospinal fluid (CSF)

Constrained spherical deconvolution (CSD)

CPL: Characteristic Path Lenth

Default mode network (DMN)

Diagnostic and Statistical Manual of Mental Disorders (DSM)

Diffusion Tensor Imaging (DTI)

Diffusion weighted imaging (DWI)

Diffusion- weighted MRI (dMRI)

Dopamine (DA)

Dorsomedial prefrontal cortex (DMPFC)

Dorsolateral prefrontal cortex (dlPFC)

Dorsal anterior cingulate cortex (dACC)

Dorsal attention network (DAN)

Drinking motive questionnaire revised short form (DMQ-R SF)

Drinking only (DRN)

Eglobal: Global Efficiency

Executive control network (ECN)

FA: Fractional Anisotropy

False Discovery Rate: FDR

Female (f)

Functional MRI (fMRI)

Functional connectivity (FC)

Frontoparietal network (FPN)

Gamma aminobutyric acid (GABA)

Glutamate (Glu)

Hamilton Depression Rating Scale (HDRS)

High angular resolution diffusion-weighted imaging (HARDI)

Independent Component Analysis (ICA)

Inferior frontal cortex (IFC)

Intercranial volume (ICV)

Male (m)

Magnetic resonance imaging (MRI)

Medial prefrontal cortex (mPFC)

Mesoparalimbic network (MPN)

National Institute on Drug Abuse (NIDA)

N-methyl-D-aspartate (NMDA)

Nucleus accumbens (NAcc)

Orbitofrontal cortex (OFC),

Penn Alcohol Craving Scale (PACS)

Prefrontal cortex (PFC)

Putamen (Put)

Positron emission tomography (PET)

Radio frequency (RF)

Resting state functional magnetic resonance imaging (Rs fMRI)

Resting state networks (RSNs),

SCID: Structured Clinical Interview DSM-V

Seed based analysis (SBA)

Sensorimotor network (SMN)

Short Michigan Alcohol Craving Scale (SMAST)

Semi-structured assessment for the Genetics of Alcoholism (SSAGA)

Substantia nigra (SN)

Ventral attention network (VAT)

Ventrolateral prefrontal cortex (VLPFC)

Ventral palladium (VP)

Ventral Striatum (VS)

Ventral tegmental area (VTA)

Young Mania Rating Scale (YMRS)

List of Figures

List of Tables

Chapter One

Introduction

Alcohol use across a range of consumptions has been a feature of our societies for millennia (Gately, 2008), however, our understanding of the neurobiological consequences of that use is limited (Topiwala & Ebmeier, 2018). Psychiatric disorders, such as bipolar disorder (BD) have always been present in our communities (Angst & Marneros, 2001), yet our knowledge of the neurobiology of these disorders remains incomplete (Savitz, Price, & Drevets, 2018). Developing scientific understanding of the brain-based impact of alcohol use and the neural underpinning of psychiatric disorders requires methodological and theoretical advances that have only recently become available. Magnetic resonance imaging (MRI) is a valuable non-invasive technique which provides researchers the opportunity to investigate the relationship between derived imaging measures and environmental or biological variables *in vivo*. In addition, advances in network neuroscience permits the use of MRI data to model the brain as a network and to investigate patterns of structural and functional connectivity (Bullmore & Sporns, 2009). This approach allows us to undercover a deeper understanding of the principles of brain organisation and its interacting components (Medaglia et al., 2016), and how this organisation may be impacted by environmental and biological variables. This thesis capitalises on these methodological and theoretical advancements in MRI and network neuroscience to identify network-wide alterations of the brain in association with alcohol use in the presence of BD. These alterations are demonstrated in networks associated with reward and affective processes, which suggest that alcohol use in BD may contribute to a vulnerability to mood lability and therefore relapse in the disorder.

Alcohol use is highly prevalent within Irish society; approximately 75% of the community consumed alcohol in 2017, with 6.7% of this figure reaching criteria for an alcohol use disorder (AUD) (Department of Health, 2018). In 2019, people aged 15 years and above consumed on average 10.8 liters of pure alcohol each, which corresponds to 40 bottles of vodka or 113 bottles of wine (O'Dwyer et al., 2021). Over half of these alcohol consumers reached criteria for hazardous alcohol use, with high rates of binge or heavy episodic drinking, particularly within men of all ages (O'Dwyer et al., 2021). A recent Irish study demonstrated that moderate alcohol consumers who binge drink at least monthly accounted for the most of the total alcohol consumed (70%) (O'Dwyer et al., 2019). The majority of risks associated with alcohol use, for instance interpersonal, physical or legal risks are accounted for by these alcohol consumers (O'Dwyer et al., 2019). This is due to most harms being experienced while intoxicated, as intoxication is most frequent within this population they account for the majority of harms experienced (O'Dwyer et al., 2019). Alcohol is also associated with a range of cancers, cardiovascular disease, liver disease, 3.7% of all deaths between 2008- 2017, and 30% of all self-harm cases in 2018 (O'Dwyer et al., 2021).

The consumption of alcohol is implicated in the development of mild cognitive impairment and Alzheimer's Disease, however, despite the neurobiological origins of these diseases, the impacts of alcohol use on the brain remain unclear (Topiwala & Ebmeier, 2018). There are identified differences in the structure, function and topology of brain networks in BD, however, there is no coherent alignment on the brain-based impact of the disorder with a variety of studies uncovering a range of differing alterations (Nabulsi et al., 2019; Syan et al., 2018; Hibar et al., 2017). Moreover, whether these alterations precede the onset of the disorder, are a consequence of the disorder or are related to treatment is poorly understood (Savitz, Price, & Drevets, 2018). Psychiatric disorders such as BD are often comorbid with alcohol use disorder (AUD) (Hunt et al., 2016), while non-dependent use is linked to a poorer

clinical trajectory and relapse in the disorder (Goldstein et al., 2006; Gordon-smith et al., 2020). Identifying the neural impacts of non-dependent alcohol use, within the range of consumptions most prevalent in society is imperative to advance our understanding of a modifiable risk to health. Moreover, understanding the biological impacts of alcohol use within BD may point to neurobiological vulnerabilities to relapse. In addition, illuminating potential biomarkers of BD in the presence of alcohol use may be beneficial in diagnostic and treatment outcomes within the disorder.

1.1 A Brief Introduction to MRI

The MR signal is derived from individual protons in the nuclei of a positively charged hydrogen atom (Currie et al., 2013). The proton spins, or precesses around its axis, which creates an electrical current that is accompanied by its magnetic moment (Schild, 1990). The magnetic moments are orientated in random directions, however, the introduction of in an external magnetic field (B0) in an MRI machine causes the protons to align parallel or anti-parallel with the external field (the z axis), the majority will align in parallel to B0 (Currie et al., 2013). The sum of the magnetisation along the z axis is referred to as longitudinal magnetisation (Smith & Webb, 2011). To create a signal RF pulses are then switched on and off, so that the protons fall out of alignment with the B0. Utilising the Larmor equation the RF required for protons to pick up energy from the radio waves is calculated, hence the term 'resonance' in MRI (Schild, 1990).

Larmor equation:

$$\omega_0 = \gamma B0$$

Where ω is the precession frequency measured in MHz, γ is the gyro-magnetic ration, and B0 is the strength of the external magnet given in Tesla (Smith & Webb, 2011). The equation for the Larmor frequency was introduced by the Irish physicist Joseph Larmor who taught in

Queens College Galway, an early iteration of the National University of Ireland Galway (Mourino, 1991). The RF pulses cause an overall reduction in longitudinal magnetisation and cause the protons to precess in phase with each other (Currie et al., 2013). This results in transverse magnetisation which creates a new vector in the x-y plane (Schild, 1990). The transverse magnetisation is a moving magnetic field which induces an alternating voltage across a receiver coil, resulting in the generation of an electrical current. This phenomenon is a manifestation of Faraday's Law of Induction where the electric charge occurs as a result of the magnetic signal and is proportional to the change in magnetisation which can be picked up as an MR signal (Smith & Webb, 2011). Once the RF is switched off the protons again align with the B0, referred to as longitudinal relaxation, the time it takes for the longitudinal relaxation to return to its individual value is called longitudinal relaxation time or T1 (Schild, 1990). Removing the RF also causes the protons to dephase, or move back to their own precession frequencies, the time it takes for transverse magnetisation to decay is referred to as the transverse relaxation time or T2.

1.2 Mapping the Human Brain

1.2.1. *Structural T1-Weighted Imaging.* Structural MR images acquired display contrasts between tissues determined by signal intensity, which in turn depends on the T1 and T2 relaxation times of the tissues within the image (Smith & Webb, 2011). A T1-weighted image refers to the difference in signal intensities due to the differences in T1 relaxation time, which are generated by manipulating the time between two RF excitation pulses, referred to as the repetition time. In a T1-weighted image the grey matter appears greyer, the white matter whiter, and the cerebrospinal fluid (CSF) dark. A T1-weighted image is a valuable methodological tool and can be used to derive such values as cortical thickness, volume, and surface area, or subcortical volumes. As the cortex develops from the inside out in a series of

columns, thickness reflects the number of neurons in a cortical column and surface area the number of cortical columns (Rakic, 1988), volume is the product of surface area by thickness (Greve, 2011). The cortex follows a distinct developmental trajectory which makes it sensitive to environmental insult (Wierenga et al., 2013), therefore relationships between alterations in cortical thickness, surface area, or volume can be investigated with reference to environmental factors for an individual. Early structural MRI work investigated volumetric differences in local areas, occasionally relating them to symptoms in a disorder (Barta et al., 1990; Shenton et al., 1992). The development of modern high resolution cortical surface area tools enabled researchers to model subject specific cortical surface, thickness, and volume, as well as subcortical volume and shape (Fischl, 2012). The harmonisation of structural methods has led to large-scale analyses being undertaken across research groups which has increased the power of researchers to compare between groups and associate with environmental or biological variables (Thompson, et al., 2020).

1.2.2 *Diffusion-Weighted Imaging.* Diffusion-weighted imaging (DWI) is a method of signal contrast generation based on the diffusion of water to study the microstructural organisation of brain tissue (Baliyan et al., 2016). The diffusion of water in the brain is less restricted along the white matter axons and tends to be anisotropic or directionally dependent, diffusion in grey matter tends to be less anisotropic, and in cerebrospinal fluid it is unrestricted in all directions (Soares et al., 2013). The '*b*-value' decides the diffusion weighting and is proportional to the square of the amplitude and the duration of the applied gradient (Baliyan et al., 2016). The modulation of the magnetic field strength along the x, y, or z coordinates spatially encodes the signal intensity in a 3D grid which can be reconstructed to obtain the signal's voxel of origin. A diffusion weighted image is created when pulsed magnetic field gradients are applied either side of the 180^{o} refocusing pulse, this introduces a

linear magnetic field inhomogeneity. The images produced by diffusion weighted MR measure the dephasing of spins of protons by comparing images with and without gradients to identify an attenuation of signal (Jones et al., 2013). The images acquired demonstrate some degree of diffusion sensitivity (le Bihan & Iima, 2015), images with high diffusion have more signal attenuation and are darker, images with low diffusion are brighter (le Bihan et al., 2006). This sensitivity can be used to investigate tissue microstructural organisation using measurement parameters such as fractional anisotropy (FA), as well as inferring the structural connectivity between regions through the recreation of white matter fibre tracts (Calamante, 2019).

Despite the pioneering work in the 1950's by Edwin Hahn, John Tanner and others, the basic principles of *in vivo* diffusion imaging were not identified until the 1980's (Le Bihan & Johansen-Berg, 2013). In 1986 the first diffusion- weighted MRI (dMRI) images of the human brain and key concepts such as the 'b factor' were published (Le Bihan et al., 1986). Despite these early strides, dMRI did not become widely used until the 1990's, due to the influence of motion distortion on images and the length of acquisition time (Le Bihan, 2011). It was not until the advent of echo-planar imaging that diffusion imaging gathered speed and suffered less from motion distortion (Le Bihan & Johansen-Berg, 2013). Diffusion phenomena were used to develop a method called Diffusion Tensor Imaging (DTI) (Basser et al., 1994) which allowed for the modelling of complex neuroanatomical information (Tournier et al., 2011). Further advances were made in the late 2000's to address limitations in the DTI model to resolve crossing fibres within a voxel, the development of high angular resolution diffusion-weighted imaging (HARDI) paved the way for constrained spherical deconvolution (CSD) which improved the modelling of fibre tracts in the brain (Tournier et al., 2008). These technological advances created models of white matter that were aligned with the known neuroanatomy, as demonstrated by *post-mortem* dissection (Catani, 2011).

1.2.3. Functional Magnetic Resonance Imaging. Functional imaging capitalises on the fact that as brain activity increases, so too does local blood flow (Poldrack et al., 2011). This phenomenon is well known and has been studied for over 100 years: in 1878 Italian neurophysiologist, Angelo Musso demonstrated an increase in cerebral blood flow in the right prefrontal cortex of a patient during a mathematical task (Raichle, 2009). This study was cited by Roy and Sherrington as evidence that cerebral blood flow can be altered by intrinsic bodily functions and extrinsic cognitive demands (Raichle, 2009; Roy & Sherrington, 1890). Localised increases in arterial blood flow due to brain activity lead to an increase in oxygenated blood and a decrease in deoxygenated blood (Poldrack et al., 2011). The MRI signal is sensitive to the difference in haemoglobin in the blood, oxygenated and deoxygenated forms of haemoglobin interact with the magnetic fields differently and induce local field inhomogeneities that depend mainly on the deoxygenated blood resulting in small but detectable changes in the MR signal (Jenkinson & Chappell, 2018). The most commonly used fMRI method is referred to as the Blood Oxygen Level Dependent (BOLD) contrast which exploits the oxygen dependent magnetic susceptibility of haemoglobin (Bandettini, 2020). It is important to note that the BOLD contrast is based on haemodynamics and is therefore a surrogate of neuronal activity (Kim & Bandettini, 2010).

The neurocircuitry underlying the functional organisation of the brain makes it difficult to explicitly identify the source of the fMRI signal, whether from inhibitory, excitatory, neuromodulatory or top-down and bottom-up processes (Logothetis, 2008). However, research in monkeys suggests that BOLD signal is primarily affected by changes in inhibition-excitation balance, which is most likely controlled by neuromodulation rather than the spiking rate of neurons and is strongly impacted by attention (Goense & Logothetis, 2008; Logothetis, 2008; Logothetis et al., 2001). Despite these limits to fMRI, it remains the

best source of information for whole brain *in vivo*, non-invasive imaging available. The use of multivariate analyses can detect small differences that are not identifiable by traditional univariate methods (Calhoun, 2018). While the use of single cell recordings may provide exquisite spatial accuracy and detail, they cannot provide information relating to the activity of cell assemblies, and the functional organisation of the whole brain, it is in these instances that fMRI comes into its own.

In 1982 Thulborn demonstrated that T2-weighted images, but not T1-weighted images were dependent on blood oxygenation and that the magnetic susceptibility difference was crucial for this effect (Thulborn, 2012). However, it was not until 1990 that the BOLD contrast was demonstrated in rats by Ogawa (Ogawa et al., 1990). The first human brain mapping evidence using fMRI was published in 1991, however, this did not make use of the BOLD contrast, instead it relied on the injection of gadolinium tracer (Belliveau et al., 1991). The year of 1992 saw three separate research groups race to be the first to publish their results in fMRI using the BOLD contrast method (Bandettini et al., 1992; Ogawa et al., 1992; Poldrack, 2018). These papers demonstrated task related activation of the brain, analysis of this data typically used a subtraction method, whereby a resting state scan was acquired and the task data subtracted from this to demonstrate where the activation occurs (Poldrack et al., 2011). The resting state data was assumed not to contain any valuable information, however, over time researchers began to identify common patterns in the resting state (Buckner et al., 2008). It was not until the seminal work of Bharat Biswal which showed that activation in the brain at rest was temporally correlated with signal across functional networks, such as the left and right motor cortex (Biswal et al., 1995).

The brain at rest reveals large amplitude spontaneous low-frequency (<0.1 Hz) fluctuations that are temporally correlated across functionally related areas (Biswal et al., 2010). The majority of energy consumed by the brain occurs during the resting state, with

task-based activations accounting for a very little increase in energy requirements (Raichle, 2009). Therefore, these intrinsic resting state activations are critical in supporting the smooth functioning of the brain. Resting state networks are found to be broadly consistent across the literature and are useful to demonstrate the functional connectivity of the brain (Laird et al., 2011; Yeo et al., 2011). As imaging methods analysis improved, functional connectivity analysis moved from seed-based approaches, which relied on the researcher determining the location of the seed, to data driven multivariate methods, such as Independent Component Analysis (ICA) methods (Calhoun et al., 2003). The characterisation of intrinsic connectivity of the brain is necessary for a more nuanced and systematic understand of the biological basis of brain function (Bressler & Menon, 2010).

1.3 The Brain as a Network

Theories of localisation of brain function can be found as far back as the 1790s with the popularity of Franz Gall's phrenology (Cobb, 2021). An elegant retort to the theory of localisation of function was delivered by Gottfried Leibniz, who believed that by focusing on parts we cannot understand the whole: he used an analogy of a mill, that on entering we may see a cog or a wheel but that will not help us understand how wheat becomes flour (Cobb, 2021). Taken forward, the Leibniz Mill argument is often used to support a network view of neuroscience, that until we approach the brain as an interacting network, we will not be able to understand or appreciably describe it (Bassett & Gazzaniga, 2011). By investigating the brain as a network, we may be able to infer areas that work together to give rise to symptoms in psychiatric disorders; networks which are compromised in association with alcohol use; and how BD and alcohol use converge on specific network vulnerability giving rise to mood lability and relapse in the disorder.

The brain is a complex system: it is comprised of components whose interactions are nonlinear, are organised hierarchically in the absence of any centralised control, giving rise to emergent behaviours, which means that the behaviour can only be understood in terms of the component interactions (Turkheimer et al., 2020). Brain networks can be described structurally or functionally, structural network organisation is based on the white matter anatomical connectivity (Bullmore & Sporns, 2009). Functional connectivity refers to the coactivation of spatially independent brain regions in the resting state or in association with a behavioural or cognitive task (Bressler & Menon, 2010). Patterns of structural connectivity constrains the functional repertoire that it can give rise to, the topology of the network is unique to each individual and is shaped by maturational and learning processes (Bressler & Tognoli, 2006). The complexity of this composition results in hierarchical networks enabling a dynamic and versatile computational architecture, ensuring appropriate behavioural responses to changing internal and external stimuli (Mesulam, 1990; Sporns, 2018). A central tenet of network neuroscience is the absence of one-to-one correspondence between anatomy, computation and behaviour, this drives the field towards descriptions of distributed and interactive networks, which are also coarse and degenerate (Mesulam, 1990). As a complex system, traditional locationist approaches of mapping dysfunctional cognitive and psychological processes associated with psychiatric disorders onto individual brain regions is implausible (Menon, 2011). Additionally, this distributed and degenerate mapping is advantageous for complex and rapid information processing and allows for flexibility of responses and adaptability in rapidly changing environments (Sporns, 2012a). Function and resultant behaviour, therefore, is not contained within a neuron or single anatomical site but is an emergent feature of patterns of connectivity that are both localised and distributed (Mesulam, 1990).

The concept that connectivity and function are predicated upon interacting circuits is not a modern idea, however, technological limitations in early MRI research pushed the field towards mapping psychiatric symptoms onto local regions. However, it is the complexity of network interactions that give rise to the constellation of symptoms which may ebb and flow in many disorders, therefore a network approach may provide richer understanding of the confluence between brain and mind (Menon, 2011). New network-based approaches provide us with information that could not have been derived using previous methods, thus in approaching the brain as a complex network and utilising fundamental principles that have been previously applied to dynamic networks, we are observing new phenomena, rather than explaining already observed information (Medaglia et al., 2016). This approach has the potential to identify mechanisms, that is the organisation of activities and entities that are responsible for the phenomena under study (Medaglia et al., 2016), for instance the brain impacts of alcohol which may inform mood lability in BD. Representing the brain as a network provides advantages in applying a unified mathematical framework to quantifying interactions, as well as examining higher order multivariate patterns rather than pair-wise interactions (Medaglia et al., 2016; Sporns, 2013). Thus, combining network methods with modern high resolution MR imaging we can further our understanding of the specific patterns and interactions underlying structural and functional connectivity in BD, in association with alcohol use, contributing to vulnerability to relapse.

1.4 Alcohol's Mechanisms of Action

Alcohol is readily miscible in water, and has significant lipid solubility, it can therefore easily cross cell membranes (Koob, 2011). Early hypotheses proposed that alcohol acted by perturbing membrane lipids, however, much evidence has since demonstrated that proteins are impacted by alcohol's action (Harris et al., 2008). Low to moderate doses of

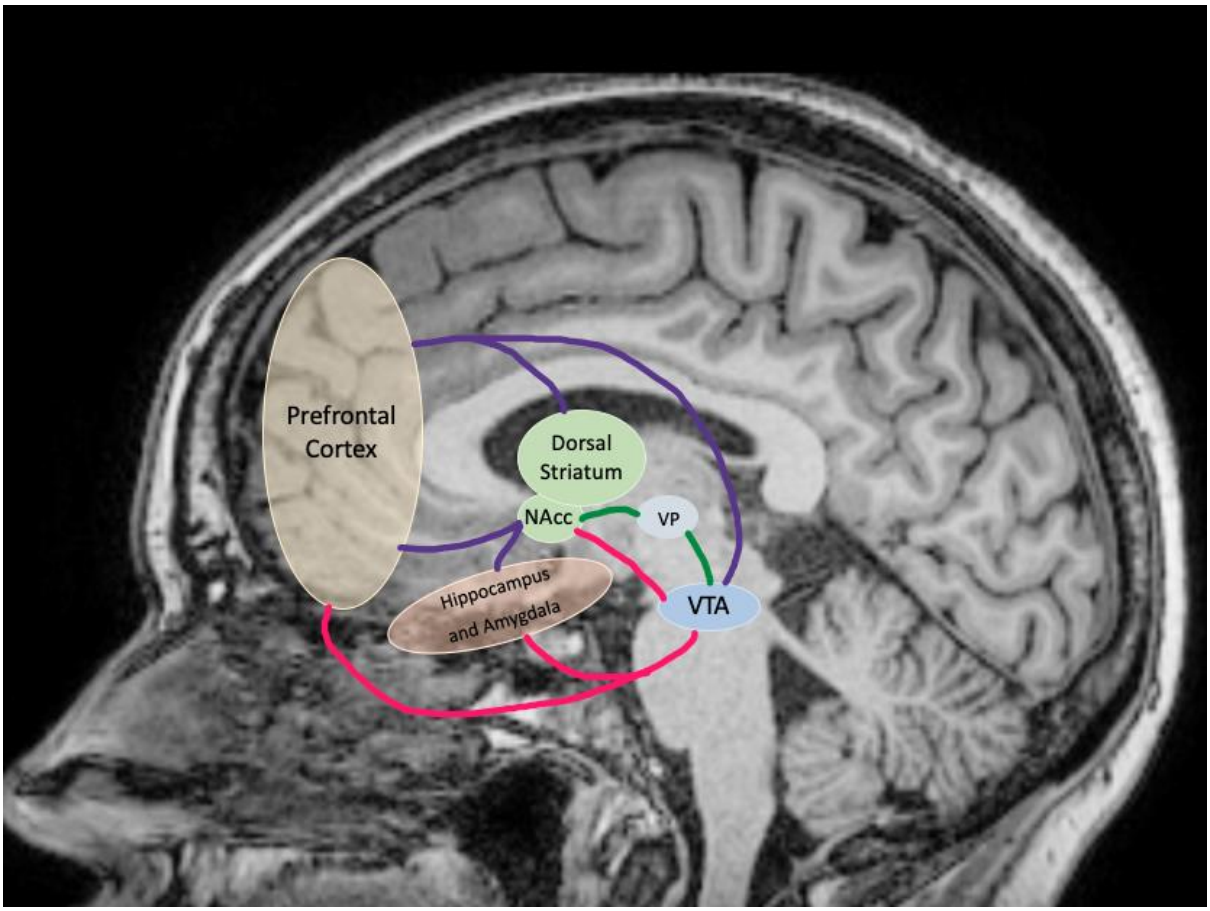
alcohol effect numerous sites in the nervous system, acting directly on the Gamma aminobutyric acid (GABA) receptor complex, serotonin receptors, and glycine receptors, as well as inhibiting glutamate receptors, potassium channels, and G-proteins (Koob, 2011). Following these first effects of alcohol on receptor sites, a second wave of indirect effects on a variety of neurotransmitter systems is initiated, with functional effects ranging from disinhibition to sedation (Spanagel, 2009). Moreover, the metabolic products of alcohol can exert a variety of actions: acetaldehyde the first product generated during alcohol metabolization contributes to the toxic effects of alcohol and leads to neuronal degeneration (Nutt, et al., 2021).

In 1989 David Lovinger demonstrated that alcohol inhibited the function of N-methyl-D-aspartate (NMDA) receptors in the hippocampus, several alcohols were tested, their inhibitory potency was related to their potency in producing intoxication (Lovinger et al., 1989). This suggested that inhibition of the responses generated by the NMDA receptor contributed to intoxication (Lovinger et al., 1989). As well as inhibitory properties, even at low doses alcohol enhances the function of GABA receptors, this contributes to a dampening of activity in the brain, a reduction in anxiety and at high doses sedation (Spanagel, 2009). Seminal work by Olds and Millner in 1954 demonstrated through electric brain stimulation that specialized networks in the brain exist for reward and reinforcement (Olds & Milner, 1954). The mesocorticolimbic dopamine system, originating in the ventral tegmental area, and projecting to limbic and cortical structures has been identified as the neurochemical substrate for the reinforcing effects of alcohol (*Figure 1.1*) (Koob, 2011; Koob & Volkow, 2016). Initial preclinical work demonstrated that low doses of alcohol increase the firing rate of dopamine neurons (Gessa et al., 1985). These findings were expanded upon to demonstrate that this increase in firing rate stimulates dopamine transmission within the mesocorticolimbic system (di Chiara & Imperato, 1988). This first quick release of dopamine

is associated with a subjective feeling of intoxication and pleasure and is necessary for rewarding effects, while triggering conditioned responses in the presence of alcohol use (Koob & Volkow, 2016). Alcohol increases extracellular dopamine through a decrease in activity of the GABAergic neurons in the nucleus accumbens, which leads to the disinhibition of mesolimbic dopamine neurons (Spanagel & Weiss, 1999). Additionally, glutamatergic activity also controls the mesolimbic dopamine pathway, at low doses alcohol may elevate extracellular glutamate, where at higher doses it reduces glutamate, demonstrating a differential effect of dose on the functioning of the system (Spanagel, 2009). These changes in receptor behaviour in the presence of alcohol use have been associated with changes in synapse plasticity, facilitating long term potentiation or inhibiting long term depression, thus altering synaptic density (Saal et al., 2003). This suggests that while alcohol use may impact the brain globally, the circuitry within the mesolimbic system in the brain may be more vulnerable to its effects (Monte & Kril, 2015).

Figure 1.1.

Circuits Regulating Dopamine Release in the Brain



Note: Various neurotransmitter systems are involved in the initial effects of alcohol and contribute to its rewarding properties.

Purple: glutamate; Green: GABA; Pink: dopamine. NAcc; nucleus accumbens; VP: ventral palladium; VTA: ventral tegmental area.

1.5 Using MRI to Identify Alcohol's Mechanistic Effects on the Brain

Balanced circuitry within the brain results in effective inhibitory control and decision making, supporting the functioning of reward, affective and interoceptive circuits, among others (Koob & Volkow, 2016). Alcohol usurps the typical functioning of this circuitry via multiple neurotransmitter specific changes to neuroplasticity (Koob & Volkow, 2016). Changes in receptor and neurotransmitter function therefore alters the structural architecture of cells through synaptic plasticity, which influences their function through changes in the connectivity of the brain. Historically, identifying these changes in the brain *in vivo* was challenging, neuroanatomical knowledge was based on *post-mortem* dissection which was limited by the skill of the anatomist and the number of specimens that could be acquired (Op de Beeck & Nakatani, 2019). Single electrode recordings, electrophysiological recordings from slices of brain tissue and electron microscopy did not exist until latter parts of the 20th Century and were limited by the invasive nature of the methods (Sullivan et al., 2010). The development and use of MRI has allowed researchers to non-invasively image the whole brain *in vivo,* without the need for biopsy or tissue preparation, moreover, the acquisition of data on a population of people is possible, making group analysis achievable (Afzali et al., 2021). While *post-mortem* dissection and electron microscopy can provide exquisite detail of cellular structure, the millimetre resolution of MRI provides statistical descriptions of the tissue, making comparisons between groups or across populations feasible (Afzali et al., 2021).

The cortex develops *in utero* from the inside out in a series of columns, the thickness of these columns depends on the number of neurons within each column (Rakic, 1988). Differential cell death or reduction in synaptic density attributable to environmental or biological factors will impact the thickness of the column (Rakic, 1988). T1-weighted structural MRI can be used to infer this cortical thickness, potentially illuminating areas

which have undergone tissue loss. Measures of cortical thickness can be undertaken across the whole brain or in specific regions of interest, and are useful to demonstrate at high resolution alterations within the grey matter of the brain. Therefore, this method can be used to identify differences in cortical thickness of grey matter that may be associated with alcohol use. Moreover, this method can also be used to identify the compound effects of alcohol use in the presence of BD on cortical thickness.

Diffusion-weighted imaging (dMRI) can be used to infer white matter fibre connectivity by delineating anatomically relevant fibre pathways using tractography based methods (Yeh et al., 2020). Moreover, dMRI can be used to derive the microstructural organisation of white matter through measures such a fractional anisotropy (FA) which may be influenced by the myelination of the axon, axonal density, or membrane permeability (Jones, Knösche & Turner, 2013). The reconstructed whiter matter tracts can be weighted by the underlying microstructural organisation of the fibre bundle and then analysed using graph theory measures (Bullmore & Sporns, 2009). This has the potential to identify between groups differences in the topological organisation of the brain globally and for subnetworks, which can be associated with environmental or biological factors. Structural connectivity appears to be under stronger genetic control than functional connectivity, this is not surprising as genes influence the size and shape of the brain (Naqvi, et al., 2021). However, genetic influence is not uniformly distributed across the brain with differing regions appearing to be under stronger genetic control (Arnatkeviciute, et al., 2021). This suggests that alcohol and bipolar disorder may have differing impacts across numerous circuits of the brain, subnetwork analysis may be useful in identifying anatomical networks that are vulnerable to compound alteration in the presence of both.

Neuroanatomical structure constrains but does not define the function of the brain, regions that are coherently active are typically connected by a direct white matter pathway

(Bassett and Gazzaniga, 2011). However, understanding the underlying organisational principles of the brain does not identify the functional activity, for this another method of MRI is required: functional MRI (fMRI). At rest, the brain exhibits large amplitude spontaneous low-frequency (<0.1 Hz) fluctuations that are correlated in time across regions (Biswal et al., 2010). These fluctuations can be decomposed into identifiable networks that are relatively stable across indivdiuals and time (Biswal et al., 2010). These intrinsic networks may be used as a tool to identify clinical biomarkers of psychiatric disorders, neuropathological progression or to predict the development of neuropathology (Zhang et al., 2021). Acquisition of resting state fMRI data does not require cognitive demands of the participant and can be used across a broad range of clinical groups (Zhang 2021). Moreover, the use of multivariate analysis in resting state fMRI is more statistically sensitive to alterations in connectivity within or between networks (Calhoun, 2018). Therefore, it is possible to move from structural alterations to identify functional changes in the brain that may be present in the context of alcohol use in BD.

The application of various MRI modalities allows this thesis to identify alterations to grey or white matter structure, as well as functional connectivity in association with alcohol use for people with a diagnosis of BD. These alterations may indicate the actions of the toxic properties of alcohol reflected in reductions in cells numbers, changes in synaptic density, and rerouting of structural and functional connectivity patterns. Moreover, conceptual and technological advances giving rise to network neuroscience provide novel tools and language to describe the structural and functional interactions of the brain (Sporns, 2012b).

1.6 Using MRI to Investigate Alcohol's Structural and Functional Effects on the Brain

Using a range of MRI methods there has been limited research into the neurobiological impacts of alcohol use, with a lack of consistency between results. Whole

brain analyses have demonstrated reduced grey matter volume and thickness globally with increased ventricle size (Immonen et al., 2020; Lange et al., 2016; Paul et al., 2008; Taki et al., 2006). Frontal, parietal, and temporal areas of the brain appear to be impacted, with regions of the anterior cingulate (ACC), orbitofrontal (OFC), dorsolateral prefrontal cortex (dlPFC), and insula particularly affected (Heikkinen et al., 2017; Kubota et al., 2001; Mashhoon et al., 2014; Meda et al., 2017; Morris et al., 2019; Sun et al., 2018). Differential impacts within the hippocampus have been demonstrated in large-scale studies; alcohol use as measured longitudinally and MRI at one time point in a sample of older UK participants demonstrated that hippocampal atrophy was associated with alcohol use (Topiwala et al., 2017). However, in a larger sample an association between alcohol use and hippocampal volume was found only for participants with depressive symptomatology (Naglich et al., 2018). This may suggest that alcohol use interacts with vulnerability for depressive symptoms to impact the brain.

Despite these findings, some studies have demonstrated no association between alcohol use and global grey matter or white matter volumes (de Bruin et al., 2005; Preti et al., 2014; Sasaki et al., 2009). Moreover, other studies have reported a protective effect of alcohol use on grey and white matter, and may also be associated with improved cognitive functioning (Anstey et al., 2006; Davis et al., 2014; Downer et al., 2015; Gu et al., 2014; Heijer et al., 2004; McEvoy et al., 2018; Sachdev et al., 2008). Discrepancies between studies may be due to methodological limitations in earlier research: for instance, reliance on coarse measures of brain structure, a variety of tools to measure alcohol use, varying power within the studies due to sample size, and the demographic and clinical characteristics of the samples. Participants who are current non-drinkers may abstain from alcohol use due to previous problematic use, their current drinking status belies the impact of their previous alcohol use on brain structure. Recent data (N=10,143) has demonstrated that even low

amounts of alcohol consumption is associated with reduced global grey matter, as well as in the cingulate and orbital frontal cortices, the bilateral insula, and thalami, in addition to negative impacts within the heart and liver (Evangelou et al., 2021). Further studies have demonstrated that the white matter of the brain is impacted by low levels of alcohol use, with the largest effect sizes demonstrated within the fornix, corpus callosum, anterior corona radiata and the left inferior longitudinal fasciculus (Daviet et al., 2021; Topiwala et al., 2021).

Studies utilizing functional MRI have demonstrated that connectivity within the brain is altered in association with non-dependent alcohol use. Seed based analyses show alterations of connectivity between the ACC and the amygdala, the striatum, and the OFC, as well as reduced connectivity between the amygdala and OFC, and between the inferior frontal cortex and hippocampus (Arienzo et al., 2020; Crane et al., 2018; Hu et al., 2018). Data driven approaches have demonstrated alterations of between network connectivity for the salience, reward, and visual networks, as well as within network connectivity changes in executive control, attention and default mode networks, in a variety of age groups from emerging to late adulthood (Mayhugh et al., 2016; Sousa et al., 2019; Vergara et al., 2017).

Taken together, this suggests that while the brain-based impacts of alcohol use are widespread, there are particular areas, nested within networks related to reward, cognitive and affective processing that may be more vulnerable to alterations. Changes to frontal areas of the brain are suggested to relate to an inability to maintain top-down cognitive control over subcortical areas and within AUD is suggested to increase the likelihood of reverting to habitual behaviours and relapse (Rando et al., 2011). Additionally, areas of the ACC, OFC, dlPFC and insula are implicated within reward circuitry, mediating and reinforcing the pleasurable experience of alcohol use (Koob & Volkow, 2016). Moreover, the smooth switching between default mode and executive control networks is suggested to underlie discreet cognitive processes and underlie successful recognition and response to emotional

stimuli (Bassett & Gazzaniga, 2011). Therefore, in the context of non-dependent alcohol use, alterations in structure and function of these networks could contribute to a dysregulation of cognitive and affective control to which some may be more vulnerable than others.

1.7 A Clinical Overview of Bipolar Disorder

Bipolar disorder is a lifelong illness which presents consistently across cultures and genders, it is associated with a variable presentation and course, loss of function and increased risk of suicide (Grande et al., 2016). A diagnosis of BD I requires only the occurrence of a manic episode with symptoms present for more than one week (Kaltenboeck et al., 2016). A diagnosis of BD II requires at least one hypomanic episode and one depressive episode. The disorder is characterised by (hypo)manic and depressive episodes, manic episodes are generally indicated by increased energy, reduced sleep requirements, racing thoughts, pressurized speech and expansive mood, depressive episodes are often characterised by decreased energy, hypersomnia, and low mood (Grande et al., 2016). Hypomania is a milder form of mania and may not be as enduring as a manic episode, mixed episodes may also present where a person experiences both mania and depression in one episode (Vieta et al., 2018). Psychosis can be experienced during either mood state; however, it is more often associated with manic periods (Vieta et al., 2018).

While a diagnosis of BD is often associated with creativity and professional accomplishment, daily function is also significantly impacted through cognitive processes, for instance control of affective and reward processes, and memory functions are disrupted within the disorder (Redfield Jamison, 2011; Vieta et al., 2018). The heterogenous presentation of the disorder, and lack of biomarkers can often lead to misdiagnosis early in presentation, the most common misdiagnoses are major depression, schizophrenia, substance abuse or anxiety (Grande et al., 2016). This can lead to a delay of 5-10 years in accurate

diagnosis and result in treatment courses which do not efficiently or effectively address the disorder (Grande et al., 2016). Environmental and lifestyle issues can impact on the severity and trajectory of the disorder, with substance use and stressful life events particularly associated with adversely impacting on treatment outcomes and time to recovery (Malhi et al., 2009). Additionally, a higher prevalence of substance use in people with multi-episode BD compared with first episode BD, is suggestive of a progressive disturbance of reward and cognitive control process in the disorder, which predisposes the person to vulnerability to habitual substance use leading to mood lability and relapse (McIntyre et al., 2020).

The heterogeneity of the clinical presentation of BD and variability of treatment course suggest that rather than one disorder, BD may represent a spectrum of disorders (Mason et al., 2016). Diagnosis of BD is based on reported symptoms and clinical observation which can lead to incorrect diagnoses and treatment plans, with a diagnosis of BD often delayed for some years (Vieta et al., 2018). Therefore, technological advances in neuroimaging can provide insights into biomarkers of the disorder and move diagnosis and treatment to a more biologically based system, thus teasing apart the spectrum of disorders and providing efficacious treatment options to patients. While biomarkers of the disorder are required to improve diagnosis and treatment outcomes, it is also imperative that modifiable risk factors and their biomarkers, for instance alcohol use are additionally identified.

1.8 Bipolar Disorder and the Brain Investigated with MRI

The heritability of BD is approximately 60-80%, concordance rates between monozygotic twins for BD is approximately 40-45% and 4-6% for dizygotic twins suggesting that the cause of the disorder is not wholly genetic and may rely on the influence of environmental factors (McIntyre et al., 2020). Complex region-specific changes of cortical gene expression in BD within the dlPFC, rostral prefrontal cortex (PFC), and ACC may be

involved in the genesis of symptoms within the disorder (Scarr et al 2019). Changes in gene expression expected to impact cell death and survival are found, with the largest effect sizes located in the dlPFC, some of which are related to dopaminergic activity (Scarr et al 2019). Widespread morphological and functional alterations have been demonstrated in the presence of BD, particularly within frontal, temporal and parietal areas (Maletic & Raison, 2014). However, some of the most consistent findings point to aberrant structure and function of circuitry related to reward and affective processing (Phillips & Swartz, 2014).

The pathophysiology of the disorder is proposed to arise from dysfunction of fronto-limbic networks related to the cognitive control of emotion and reward and disruption in the ability of these areas to exert a top-down control over subcortical areas which are involved in the processing of affective stimuli (Phillips et al., 2008). Widespread cortical and subcortical alterations are demonstrated in the disorder, with largest effect sizes demonstrated within structures of the corticolimbic system (Hibar et al., 2016, 2017). Additionally, largescale analysis has demonstrated widespread alterations in the microstructural organisation of white matter in the brain, with particular effect in reward and affective circuitry (Favre et al., 2019). Moreover, reduced connectivity within the medial PFC and the ventral anterior cingulate has been demonstrated in BD, this area is known to be a critical region for emotion regulation through its through afferent and efferent connections to frontal and subcortical areas (Anticevic et al., 2013). Alterations in the ACC are suggested to be related to difficulties in adapting to changes in emotional and social contexts, increased functional connectivity occurs within this area during an induced sad mood for participants with BD (Maletic & Raison, 2014; Phillips, 2003). Additionally, increased activity within subcortical structures related to emotion control, for instance the amygdala and hippocampus, have been demonstrated in BD during emotional processing tasks (Strakowski et al., 2011).

The heritability of BD points to evidence of a neurodevelopmental aetiology, further support for this is demonstrated in alterations of cortical folding in the ACC in BD (Fornito, et al., 2007). Cortical folding patterns are primarily formed by birth and are relatively stable therefore, these changes implicate pre or peri-natal developmental processes (Fornito, et al., 2007). Paediatric patients with BD demonstrate volumetric reductions of the ACC in both medicated and unmedicated cases, this suggests that volumetric reduction predates the illness onset (Chiu, et al., 2005). Moreover, increasing genetic risk for BD is associated with volume reductions in the ACC (McDonald, et al., 2004). Alterations in functional connectivity are also demonstrated within and between networks for people with a diagnosis of BD and their unaffected relatives, providing further support for a neurodevelopmental basis to the disorder (Meda et al., 2017; Khadka et al., 2013).

However, longitudinal neuroimaging studies also demonstrate progressive abnormalities within cortical and subcortical structures of the fronto-limbic network which relate to emotion regulation, as well as functional alterations related to illness phase and mood state (Lim, 2013). A recent large-scale longitudinal study determined that participants with a diagnosis of BD showed faster enlargement of ventricles as well as a slower thinning of the fusiform and parahippocampal cortices (Abe et al., 2020). Additionally, an increased number of manic episodes were associated with faster thinning in the lingual and frontal pole cortices; while increased hypomanic and mixed episodes were associated with faster thinning in frontal regions (Abe et al., 2020). Taken together, these results suggest that BD may be a neurodevelopmental disorder which is vulnerable to compound progressive alterations. These compound insults may be related to environmental factors, particularly in areas supporting affective and reward processes. Recall that people with multi-episode BD compared with first episode BD demonstrate a higher prevalence of substance use, suggesting a progressive disturbance of reward and cognitive control (McIntyre et al., 2020). Therefore, the reported

poor illness trajectory and vulnerability to relapse in BD, in association with non-dependent alcohol use may be reflected in the exacerbation of neuroprogressive alterations.

1.9 Alcohol Use in Bipolar Disorder

Prevalence rates for AUD in BD are estimated by metanalysis to be around 35% (di Florio et al., 2014), in comparison to global prevalence rates of 2.2- 5.1% (Glantz et al, 2020; Rehm & Shield, 2019). Alcohol use disorder comorbid with BD, has been associated with a negative illness trajectory characterised by increased mood episodes, higher rates of suicide attempts, a younger age of onset, and increased likelihood of further substance use disorders (Cardoso et al., 2008; Nery et al., 2014). However, while these associations are concerning, they do not encompass a complete pattern of alcohol use within BD and provide us with little information on the impact of non-dependent alcohol use within the disorder.

Research has demonstrated that women with a diagnosis of BD who consume low levels of alcohol experience more lifetime depressive and hypomanic episodes, while men experience increased manic episodes across the lifespan and more frequent hospitalizations (Goldstein et al., 2006). In contrast, a Dutch prospective follow up study demonstrated that for participants with BD who adhered to mood stabilizers, there was no association between alcohol use and lifetime clinical characteristics measured at baseline or at follow up (van Zaane et al., 2014). However, a large-scale UK sample found that increasing alcohol use, while still not reaching criteria for an AUD in people with a diagnosis of BD, was associated with increased presence of suicide attempts and rapid cycling (Gordon-smith et al., 2020). Moreover, for women, increased levels of alcohol use were associated with a greater number of depressive episodes and mania, comorbid panic and eating disorders, and was also associated with less impairment in functioning during severe mood episodes and fewer psychiatric admissions (Gordon-smith et al., 2020). These limited studies suggest that there

may be an association between alcohol use and vulnerability to relapse in BD, but this requires further research. While this thesis is limited in its ability to clearly identify factors related to relapse, the largely euthymic sample is sensitive to detect trait-features and decipher a possible unrecognized prior confound in the literature. Previous brain-based studies of BD may not have controlled for alcohol consumption, this thesis may identify that it is a factor that should be taken into consideration in further statistical models.

Research focusing on participants with comorbid BD and AUD report reduced volume of the medial frontal gyrus and anterior cingulate cortex (ACC) only in those with comorbid BD and AUD, relative to BD only and healthy controls (Nery et al., 2011). However, the comorbid group within this study were in remission from alcohol use for on average six years; almost half of the AUD-BD group reported a previous substance use disorder, therefore, generalization to the effects of AUD is questionable. Within the study design current alcohol use among the BD only group and healthy controls was not recorded, therefore a true pattern of alcohol use within the groups is unknown. One previous study demonstrated widespread cortical thinning in association with non-dependent alcohol use, this finding was present at low levels of alcohol use and did not differ between patients with a diagnosis of BD, schizophrenia, or healthy controls (Lange et al., 2016). This may suggest that non-dependent alcohol use in BD has a localised, perhaps compound impact on distinct structures within the brain that are involved in the processing of emotion and reward. Identifying and understanding the alterations within and between these areas may be more biologically informative regarding the impact of alcohol use and the specific vulnerability to mood lability established in BD.

1.10 Overview of the Thesis

While alcohol has a widespread impact on the brain, it is likely that there are specific circuitry involved in emotion and reward processes which may be more vulnerable to its toxic effects (Monte & Kril, 2015). The extant literature concerning the associations of alcohol use and bipolar disorder on the brain identify common areas of impact. These impacts are additional to typical architectural alterations found in healthy brain aging and are demonstrated structurally in frontal regions: ACC, dlPFC, insula, OFC and functionally in default mode (DMN) and frontolimbic networks (Morris et al., 2019; Guadalupe et al., 2017; Hibar et al., 2017; Lange et al., 2017, Strakowski et al., 2012). That these regions and networks subserve reward and affective processes suggests that the compound impact of alcohol use in the presence of BD may exacerbate pre-existing alterations. This indicates that while BD may be a neurodevelopmental disorder, it also is neuroprogressive in nature, and alcohol use in the disorder may contribute to alterations leading to the likelihood of mood lability and relapse. Magnetic resonance imaging is well placed to identify these compound alterations as it is non-invasive, can be used *in vivo,* it has the capacity to image large groups of people and allows for statistical comparison between groups (Afzali et al., 2021). Moreover, resting state fMRI has the potential to identify biomarkers related to BD or alcohol use that can be used to develop objective clinical markers of diagnosis and can improve treatment outcomes for patients.

This thesis uses subject specific measurements of cortical thickness within reward regions of the brain to estimate the association between alcohol use in healthy controls and those with a diagnosis of BD. The application of cortical surface reconstruction tools, which measure the MR intensity gradients at each cortical vertex between grey matter and cerebrospinal fluid, have demonstrated excellent reliability in the measurement of cortical thickness in human brains (Dale et al., 1999; Fischl et al., 1999, 2001). This method points to areas of the cortex which may experience reductions of cell density in association with alcohol use, potentially

indicating regions vulnerable to the toxic effects of alcohol use. Additionally, this thesis uses state of the art network neuroscience methods to model the brain as a graph, through the recreation of white matter tracts using deterministic nontensor-based constrained spherical deconvolution (CSD) (Tournier et al., 2008), and T1-weighted imaging, parcellated and segmented in subject-specific space using Freesurfer (v5.3.0) (Fischl, 2012).

Network neuroscience capitalises on the understanding that the behaviour of complex networks is driven by the interaction of their constituent parts (Bullmore & Sporns, 2009). It is therefore possible in modelling the brain as a graph to explore the integrative and segregative capacities of the network and also to identify subnetworks which are associated with alcohol use in BD (Sporns, 2018). This topological analysis provides richer information on the structural architecture of the brain and how alcohol use may impact the connectivity of the network. In applying these methods this thesis has the potential to contribute new knowledge on the structural organisation of the brain in association with alcohol use and in the presence of BD.

Lastly, developments in methodology and advances in knowledge have allowed intrinsic fluctuations in the resting state to be decomposed and mapped onto behavioural and cognitive functions (Laird et al., 2011). This thesis makes use of independent component analysis (ICA) methods to decompose resting state fMRI data into intrinsic networks of the brain. Using this multivariate approach can more sensitively identify weak contributions from numerous regions contributing to intrinsic network alterations (Calhoun, 2018). This method avoids the locationist approach of seed-based analysis and uses whole brain functional connectivity to identify alcohol related alterations to within and between network connectivity in BD.

1.11 Aims and Thesis Outline

This thesis utilises high resolution neuroimaging and network neuroscience methods with the overall aim of identifying alterations in the structure and function of the brain in BD in association with alcohol use. Combining a number of MRI methodologies allows the investigation of brain networks across multiple spatiotemporal scales, that of global and mesoscale network organisation in structure and function. The thesis design is observational and cross-sectional in nature, therefore it can report on associations within the data and cannot infer causation. The overall hypothesis is that alcohol use would be associated with structural and functional alterations, with differential effects in participants with a diagnosis of BD, particularly within reward, emotional and cognitive networks.

Manuscript one*: Alcohol Use Impacts Cortical Reward Network Structure in Bipolar Disorder* focuses on specific cortical regions within reward circuitry which have identified alterations in association with alcohol use and BD. Alcohol use is measured using the AUDIT-C which has been validated for research use (Bush et al., 1998). Measures of cortical thickness are examined for an effect of alcohol use and compared between BD and control groups covarying for age, sex and diagnosis. We propose that BD participants will demonstrate particular vulnerability to alcohol use in these specific reward related regions.

Manuscript two*: Topological Alteration is Associated with Non-Dependent Alcohol Use in Bipolar Disorder* explores anatomical connectivity utilising a network analysis to identify topological alterations associated with alcohol use in BD. This novel study measures alcohol use via the AUDIT-C and examines it against subnetwork connectivity strength and global network connectivity. We propose that alcohol use will be associated with differential

connectivity within BD in comparison to control participants, pointing to network vulnerability in the disorder.

Manuscript three: *Alcohol Use is Associated with Affective and Interoceptive Network Alterations in Bipolar Disorder* examines intrinsic functional connectivity in relation to alcohol use in BD to provide further context to the neural basis of vulnerability to relapse in BD. A data driven approach of independent component analysis is used to decompose fMRI data into intrinsic networks, alterations in within and between network structure are associated with alcohol use as measured by the AUDIT-C. We propose that alcohol use will be associated with alterations to networks related to reward, emotion, and self-referential processes, with compound effects in BD.

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Chapter Two

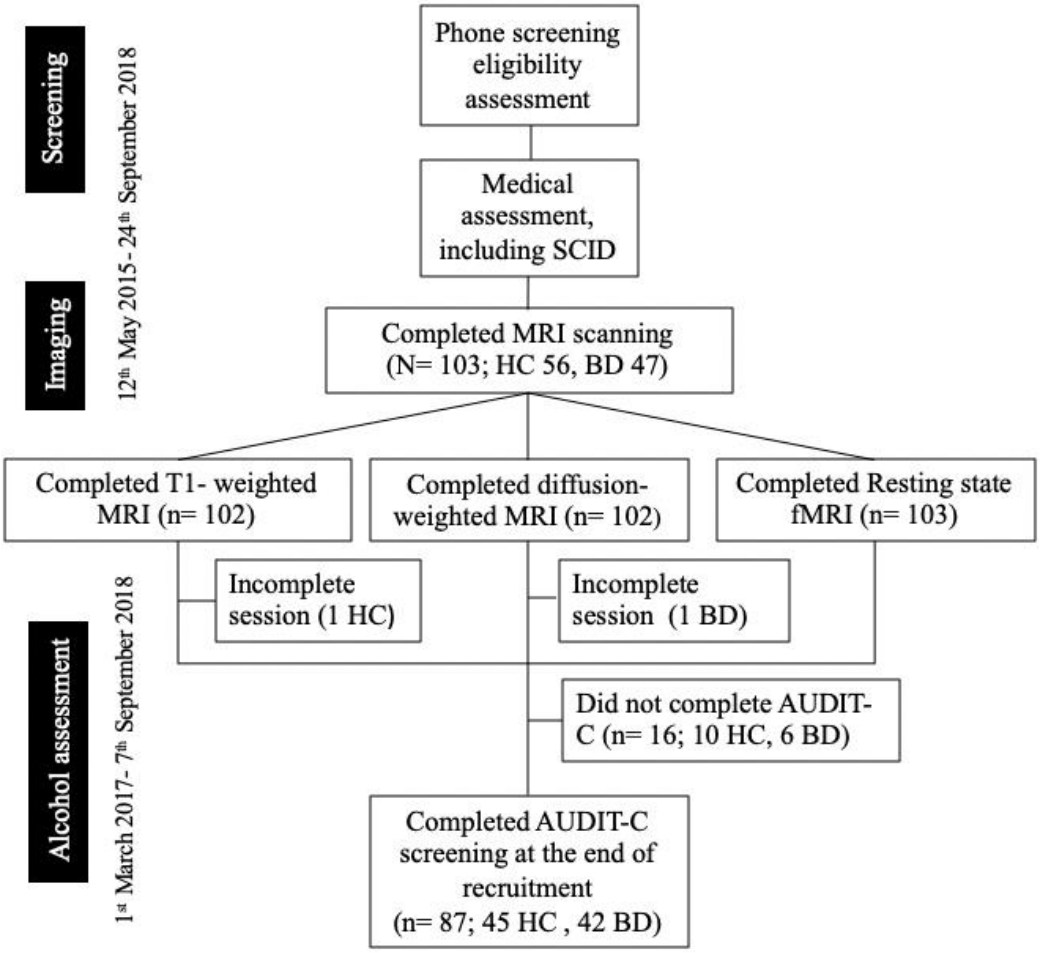
Thesis methods

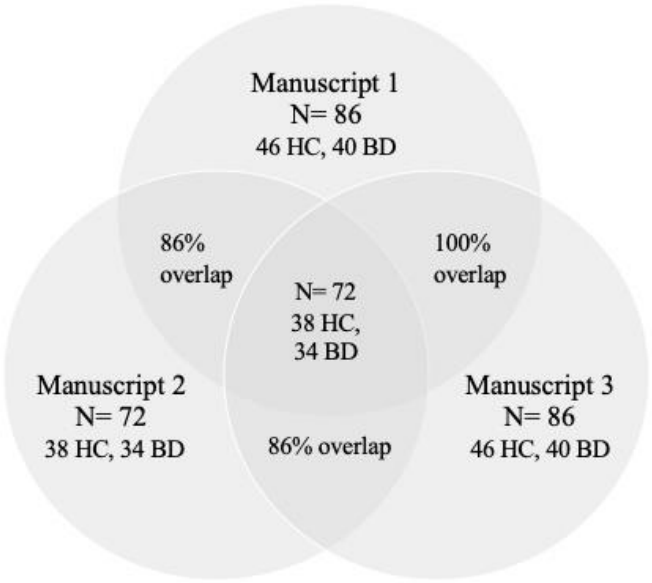
The participant data used in this thesis was collected as a part of the '*Genetic variation in the muscarinic cholinergic M2 receptor gene and cholinergic neurotransmission in bipolar disorder'* study. This project was funded by the Health Research Board grant (HRA-POR-324) awarded to Dr Dara Cannon. Recruitment for the study was undertaken primarily through psychiatry services in the Western Region of Ireland, with some additional recruitment through radio and newspaper advertisements, and through word of mouth. Study participation involved an initial phone call to assess eligibility for inclusion, and three subsequent visits, the first for clinical and medical assessments, the second for MRI scanning, and the third for cognitive testing. The first and the third meetings took place at the University Hospital Galway, the second imaging appointment acquired MRI scans using a 3T Philips Achieva machine at the Centre for Advanced Medical Imaging (CAMI), St James' Hospital, Dublin. This thesis uses T1 and diffusion-weighted imaging, and resting state functional imaging, each manuscript describes the acquisition and pre-processing parameters in detail. All participants met with a psychiatrist to undergo a Structured Clinical Interview for DSM-IV-TR (SCID) (American Psychiatric Association, 2000), either patient or control versions. For BD participants this interview confirmed a diagnosis of BD, for all participants the interview assessed suitability for the study, exclusion criteria were: neurological disorders, intellectual disability (intelligence quotient < 70), comorbid lifetime diagnosis of alcohol or abuse or dependence, history of head injury resulting in a loss of consciousness (>5 minutes), or any other illness potentially affecting cognitive function. The SCID confirmed that participants were not comorbid for a lifetime diagnosis of alcohol or abuse or dependence, however, this assessment was not used to quantify alcohol use. Alcohol use was

assessed as a scalar value using the validated and reliable Alcohol Used Disorders Identification Tool- Consumption (AUDIT-C) (Bush, Kivlahan, McDonell, Fihn, & Bradley, 1998), this tool was added to the study battery after study commencement following additional ethical approval. Alcohol use was assessed between 0-21 months post MRI scan, there was no difference in times between scans and assessment between the bipolar participants and healthy controls. Figure 2.9 outlines the study protocol and numbers of participants who completed each visit and were included in subsequent manuscripts.

Figure 2.1

Flowchart Depicting the Overall Study Design and the Data Included in Each Study in This Thesis





Chapter Three

Study One: Alcohol Use Impacts Cortical Reward Network Structure in Bipolar Disorder

Author list: Fiona M. Martyn[1;2], Genevieve McPhilemy[1], Leila Nabulsi[1,3], Theophilus N. Akudjedu[1,4], Giulia Tronchin[1], James McLoughlin[1], Brian Hallahan[1], Colm McDonald[1], & Dara M. Cannon[1].

Affiliations:

[1]Centre for Neuroimaging, Cognition and Genomics (NICOG), Clinical Neuroimaging Laboratory, NCBES Galway Neuroscience Centre, College of Medicine, Nursing, and Health Sciences, National University of Ireland Galway, H91 TK33 Galway Ireland.

[2]School of Psychology, National University of Ireland Galway, Galway Ireland.

[3]Imaging Genetics Center, Mark and Mary Stevens Neuroimaging & Informatics Institute, University of Southern California, Marina del Rey, CA 90292, USA, Los Angeles, CA, United States.

[4]Institute of Medical Imaging & Visualisation (IMIV), Department of Medical Science & Public Health, Faculty of Health & Social Sciences, Bournemouth University, UK.

Authorship Confirmation Statement

FMM contributed to recruitment and data collection, performed MRI data quality checks, conducted all analyses and wrote the manuscript. DMC designed, obtained funding for and supervised data collection, analysis, and interpretation; JMcL, BH and CMcD contributed to

recruitment and intellectual content; LN and GMP recruited and collected data, TNA and GT contributed to intellectual content.

Submitted to *Alcohol*

3.1 Abstract

Widespread alterations to frontal, temporal and parietal cortical regions particularly in areas related to reward processes have been described following non-dependent alcohol use and independently in bipolar disorder (BD). Identifying any impact of non-dependent alcohol use within the neuroanatomical reward circuitry may aid in explaining the vulnerability to relapse in BD.

Forty-six psychiatrically healthy and 40 BD (DSM-V-TR) participants underwent T1-weighted (MPRAGE) MRI scanning at 3T, and the AUDIT-C to assess alcohol use. Regions of interest were parcellated based on the Desikan-Killiany atlas (Freesurfer v.5.3.0) and included the anterior cingulate (ACC), dorsolateral prefrontal (dlPFC), and orbitofrontal cortices (OFC), and insula. Cortical thickness was examined for an effect of alcohol use and compared between BD and control groups covarying for age, sex and diagnosis.

For all participants, alcohol was associated with reductions in cortical thickness of the left ACC (T=-2.984, p_{FDR}=0.016), left OFC (T=-2.508, p_{FDR}=0.025), and left insula (T=-2.385, p_{FDR}=0.025). The diagnostic groups consumed similar amounts of alcohol (HC: age mean±SD= 41±14; BD: 43±13) (U=-713.5, p=0.072), with BD participants only demonstrating an association between alcohol use and cortical thickness in the left dlPFC (T=-2.237, p=0.032). No difference in cortical thickness was demonstrated between the diagnostic groups.

We demonstrate an effect of alcohol use on specific areas of the reward network. Alterations in BD may contribute to compromised cognitive control and reward expectancies, leading to a dysregulation of emotion processing, accelerating the possibility of relapse within BD.

Keywords (3-10): Bipolar disorder; non-dependent alcohol use; reward; structural MRI

3.2 Introduction

Widespread alterations in frontal, temporal and parietal cortical regions and subcortical structures, particularly in those related to reward and emotion regulation have been described independently following non-dependent alcohol use (Lange et al., 2016; Topiwala et al., 2017) and bipolar disorder (BD) (Hibar et al., 2018; Hibar et al., 2016). Individuals with BD who engage in non-dependent levels of alcohol use have demonstrated a poor clinical trajectory, with increased numbers of depressive, and (hypo)manic episodes (Gordon-smith et al., 2020; Goldstein et al., 2006), therefore accelerating their likelihood of relapse in the disorder. Complex behaviours such as reward processing and emotion regulation are underpinned by interactions between cortical and subcortical regions (Koob & Volkow, 2016); thus, understanding neuroanatomical impacts associated with non-dependent alcohol use within specific circuity, may inform the mechanism of conferred vulnerability to relapse associated with non-dependent levels of alcohol use in BD. We hypothesise that alcohol use will be associated with alterations to specific neuroanatomical structures related to reward processes, with an additional biological vulnerability conferred to those with a diagnosis of BD.

The central components of reward circuitry are the basal ganglia comprising the caudate, putamen, nucleus accumbens, olfactory tubercle, and globus palladus. These structures have reciprocal connections with the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and midbrain dopaminergic neurons (Haber & Knutson, 2010). The amygdala, dorsolateral prefrontal cortex (dlPFC), insula, hippocampus and thalamus regulate the functioning of this system (Haber & Knutson, 2010). Functional magnetic resonance imaging (fMRI) has demonstrated that this circuitry is active in rewarding contexts, such that, for non-dependent cohorts, acute alcohol use is associated with reduced connectivity between frontal

and cingulate cortices, and subcortical areas (Zheng et al., 2015; Gilman et al., 2008), and for BD, hyperactivation of ventral striatum and frontal areas, and reduced activity of ACC during anticipation of reward (Mason et al., 2016). Behaviour directed by reward expectations emerges due to the dynamic interaction of all structures within this network, the functioning of individual regions is better understood in terms of their role within the extended network (Kelley & Berridge, 2002). However, whether functional alterations correspond to underlying neuroanatomical changes including thickness or volume of structures remains unclear.

Widespread changes in cortical thickness have been demonstrated in association with non-dependent alcohol use as well as reductions in regions specific to reward processes such as the ACC, dlPFC, insula, and middle frontal gyri, and subcortically with an increased likelihood of hippocampal atrophy (*Table 3.1*) (Lange et al., 2016; Topiwala et al., 2017; Morris et al., 2019; Taki et al., 2006). Moreover, binge alcohol use in college age cohorts has been associated with reductions in cortical thickness of the ACC, and posterior cingulate cortices, in addition to volumetric reductions of the ACC, insula, superior temporal gyrus, OFC, and parahippocampus gyrus (Heikkinen et al., 2017; Mashhoon et al., 2014; Meda et al., 2017). However, within a highly powered study (N=1848), non-dependent alcohol use was not associated with reductions in hippocampal volume, with *post-hoc* results demonstrating changes in the structure in those only with increased depressive symptoms.(Naglich et al., 2018). A diagnosis of BD has been associated with widespread decline in cortical thickness particularly in parietal, temporal and frontal areas of dlPFC, ACC, OFC, and insula, and within subcortical structures of the basal ganglia, hippocampus, amygdala, and thalamus (Hibar et al., 2018; Hibar et al., 2016). Taken together this suggests that not all structures involved in reward circuitry are impacted by alcohol or BD, but that there are common localised alterations within the dlPFC, OFC, ACC, insula and hippocampus. Where reported, despite being modest in magnitude, the most consistently

established effects (*Table 3.1* listing effect sizes) are found within the bilateral dlPFC, OFC, ACC, and insula (Lange et al., 2016; Hibar et al., 2018; Morris et al., 2019; Meda et al., 2017). These consistent effects are demonstrated within highly powered samples and are found across cortical thickness and volumetric studies. However, despite large sample sizes with a commonality of methods, hippocampal studies display discordant results therefore, the hippocampus is not a plausible candidate for inclusion in the current study. Despite demonstrating widespread reductions in cortical thickness in association with alcohol use, Lange *et al.* (2016) demonstrated no difference in association between healthy controls and those with a diagnosis of BD or schizophrenia. This may suggest that non-dependent alcohol use in BD has a localised, perhaps compound impact on distinct reward structures. Therefore, understanding the associations between specific areas of reward circuitry and non-dependent alcohol use in BD, rather than taking a whole brain approach, may be more biologically informative regarding the impact of alcohol use, and the specific vulnerability to relapse established in BD.

We aim to identify if cortical thickness of specific consistently implicated structures within reward circuitry is associated with alcohol use, and if this effect is additively deleterious in BD. We hypothesised that non-dependent alcohol use would be (1) associated with lowered cortical thickness of the bilateral dlPFC, ACC, OFC, and insula in the BD group relative to the control group; (2) have a dose dependent effect on cortical thickness of these structures and (3) will be associated with further lowering of cortical thickness in individuals with BD compared to the control group.

Table 3.1

Studies Reporting Neuroanatomical Alteration in Association with Alcohol Use or Bipolar Disorder

Authors	*Sample size*	*% women: men*	*Age years: mean±SD*	*Methods*	*Alcohol data*	*Findings (effect size)*
Moderate alcohol use studies						
Lange *et al,* 2016	609	48:52	34.2±9.9	Cortical thickness	AUDIT-C	↓ Rostral middle frontal (**L: 2.5% R: 1.6% ΔR^2**), ↓ Superior frontal (**L: 2.3%, R: 2.2% ΔR^2**), ↓ Insula (**R: 1.7% ΔR^2**)
Morris, *et al,* 2019	706	51:49	28.8±3.6	Cortical thickness	SSAGA	↓ Left dlPFC (**1.1% ΔR^2**)
Taki *et al,* 2006	405	0:100	47±14.6	Grey matter volume	Lifetime alcohol history	↓ Middle frontal gyri (**L: 17.3%; R: 15.3% R^2**)
Topiwala *et al,* 2017	527	35:65	43±5.4	Grey matter volume	CAGE, AUDIT	↓ Right hippocampus (16% R^2)

Naglich, *et al,* 2018	1848	59:41	49.8±10.5	Hippocampal volume	Drinks per week	↔ Hippocampal (L: 25.3%; R: 22.5% R^2)
Binge alcohol use studies						
Mashhoon, *et al,* 2014	54	48:52	22±1.2	Cortical thickness	AUDIT	↓ Right ACC (**16.8% R^2**)
Heikkinen *et al,* 2017	62	56:44	24.9±1.4	Grey matter volume	AUDIT-C	↓ Bilateral ACC (0.07), ↓ Right OFC (0.02), ↓ Right insula (0.06) †
Meda, *et al,* 2017	139	49:51	18.5	Grey matter volume	SSAGA, SCID	↓ ACC, ↓ Insula, ↓ Middle frontal cortex‡
Bipolar disorder studies						
Hibar *et al,* 2017	4419	57:43	35.4±6.6	Cortical thickness	Not collected	↓ Caudal ACC (***d*= L: -0.095; R: -0.063**) ↓ Caudal middle frontal (***d*= L: -0.266; R: -0.208**) ↓ Insula (***d*= L: -0.198; R: -0.168**) ↓ Lateral OFC (***d*= L: -0.216; R: -0.207**) ↓ Medial OFC (***d*= L: -0.199; R: -0.230**) ↓ Rostral ACC (***d*= L: -146; R: -0.0.86**)

						↓ Rostral middle frontal (***d*= L: -0.270; R:-0.264**) ↓ Superior frontal (***d*= L: -0.233; R: -0.256**)
Hibar *et al*, 2017	4304	57:43	38.4±4.9	Subcortical volume	Not collected	↓ Amygdala (***d*=** -0.108), ↓ Hippocampus (***d*=** -0.232), ↓ Thalamus (***d*=** -0.480)

Note: ACC: Anterior Cingulate Cortex; AUDIT-(C): Alcohol Use Disorders Identification Test (Consumption); *d*= Cohen's d; dlPFC: Dorsolateral Prefrontal Cortex; L: Left; OFC: Orbitofrontal Cortex; R: Right; R^2: percentage of variance attributable to non-dependent alcohol use; SCID: Structured Clinical Interview DSM-V; SSAGA: Semi-structured assessment for the Genetics of Alcoholism; ↓: decreased cortical thickness; ↔: no change in cortical thickness; ΔR^2: change in R^2 or change in percentage of variance attributable to non-dependent alcohol use; †: effect size not defined in literature; ‡: effect sizes not given in literature; §: Means displayed are mean of means

3.3 Methods

3.3.1 Participants

Psychiatrically healthy individuals and outpatients with a diagnosis of BD between 18 and 65 years of age were recruited through the mental health services in the Western region of Ireland. A diagnosis of BD was confirmed using the Structured Clinical Interview for DSM-IV-TR (SCID) (American Psychiatric Association, 2000). Healthy volunteers had no personal history of psychiatric illness (SCID non-patient edition) and no first-degree family history of psychiatric illnesses. Exclusion criteria included neurological disorders, intellectual disability (intelligence quotient < 70), comorbid lifetime diagnosis of alcohol or abuse or dependence, history of head injury resulting in a loss of consciousness (>5 minutes), or any other illness potentially affecting cognitive function. Bipolar participants were included if they had a diagnosis of BD type-I or II and were aged between 18-65 years. Participants were excluded if they had a current or lifetime alcohol or substance use disorder or dependence, or a history of head injury leading to loss of consciousness (>5 minutes), as determined during the course of the structured clinical interview. All participants provided fully informed written consent and ethical approval was granted by the Galway University Hospitals Clinical Research Ethics Committee.

3.3.2 Clinical Assessments

Alcohol Use Disorder Identification Test. The Alcohol Use Disorder Identification Test (AUDIT) consumption sub-score (frequency and amount of alcohol consumed: AUDIT-C) was used in this analysis. The AUDIT-C comprises the first three questions of the AUDIT, and addresses current frequency of alcohol use, amount of alcoholic drinks consumed, and frequency of binge alcohol drinking (≥6 standard drinks in one episode). The instrument has

excellent validation and reliability indices for use in clinical and research settings to quantify recent alcohol consumption (Dawson et al., 2005). Each question is scored from 0 to 4, with a maximum possible score of 12. A standard drink in Ireland contains 10g of alcohol, within an Irish population a score of >5 on the AUDIT-C indicates a potential for harmful use, however this cut-off can vary country to country (Long & Mongan, 2013). The AUDIT-C characterises a large range of alcohol intake, a score of one can indicate that a person consumes one to two standard drinks monthly or less, the maximum score of 12 can indicate daily or almost daily binge use of alcohol, equating to at least six standard drinks each day. The AUDIT-C was collected retrospectively after MRI brain scanning for a majority of participants and pertained to the twelve months prior to imaging. There was no difference in range of time between scan and the administration of the tool between the two groups.

3.3.3 Hamilton Depression Rating Scale

The Hamilton Depression Rating Scale (HDRS) is an objective rating instrument with excellent reliability and validity indices for the identification of depressive symptomatology (Trajkovi et al., 2011). It comprises 24 questions which identify symptoms of depression over the previous week, scoring is based on the first 17 items, with a range of 0-53, a score of <8 indicating the absence of depressive symptoms.(Hamilton, 1960)

3.3.4 Young Mania Rating Scale

The Young Mania Rating Scale (YMRS) is an objective rating instrument with excellent reliability and validity indices for the identification of (hypo)manic symptoms (Young & Meyer, 1978). It consists of 11 items; scoring is based upon objective ratings during a clinical interview. A score of <7 indicates the absence of (hypo)manic symptoms, scoring <8 in the HDRS or <7 in the YMRS indicates a euthymic state in the BD participants

(Young & Meyer, 1978) All participants completed the HDRS and the YMRS on the day of MRI screening.

3.3.5 MRI Acquisition

For all participants, high-resolution structural T1-weighted brain images were obtained using the *Magnetisation Prepared Rapid Acquisition Gradient Echo* (MPRAGE) sequence on a 3T Achieva MRI scanner (Philips Medical Systems, Netherlands) with an 8-channel head coil at the following acquisition parameters: echo time (TE)= 3.9 ms; repetition time (TR)= 8.5 ms; flip angle= 8°; for 180 slices at an isotropic voxel size of $1mm^3$.

3.3.6 MRI Processing

For cortical analysis, T1-weighted MR images were transformed to Talairach image space, corrected for motion distortions, underwent intensity normalisation, and skull stripped (Dale et al., 1999). Images underwent quality control processes to ensure correct registration of image coordinates to Talairach coordinates, accurate correction for magnetic field inhomogeneity of T1-weighted images, and the satisfactory removal of non-brain tissue without the loss of brain tissue. The images then underwent segmentation of white matter based on image intensities, tessellation of the white matter surfaces using a smoothed triangular mesh, and correction of topological defects using atlas-based segmentation (Dale et al., 1999). The resulting white matter surface was deformed outwards to estimate the local intensity gradients between the grey matter and cerebral spinal fluid, which identifies the pial surface allowing for the detection of boundaries of grey, white and pial tissue in subject specific space (Dale et al., 1999). This method of boundary detection reliably categorises the border between grey/white matter, and pial surface using the deformation of the tessellated

white matter surface to denote the edges of the grey/white matter and pial surfaces (Freesurfer v5.3.0) (Dale et al., 1999; Dale & Sereno, 1993; Fischl, 2012).

3.3.7 MRI Analysis

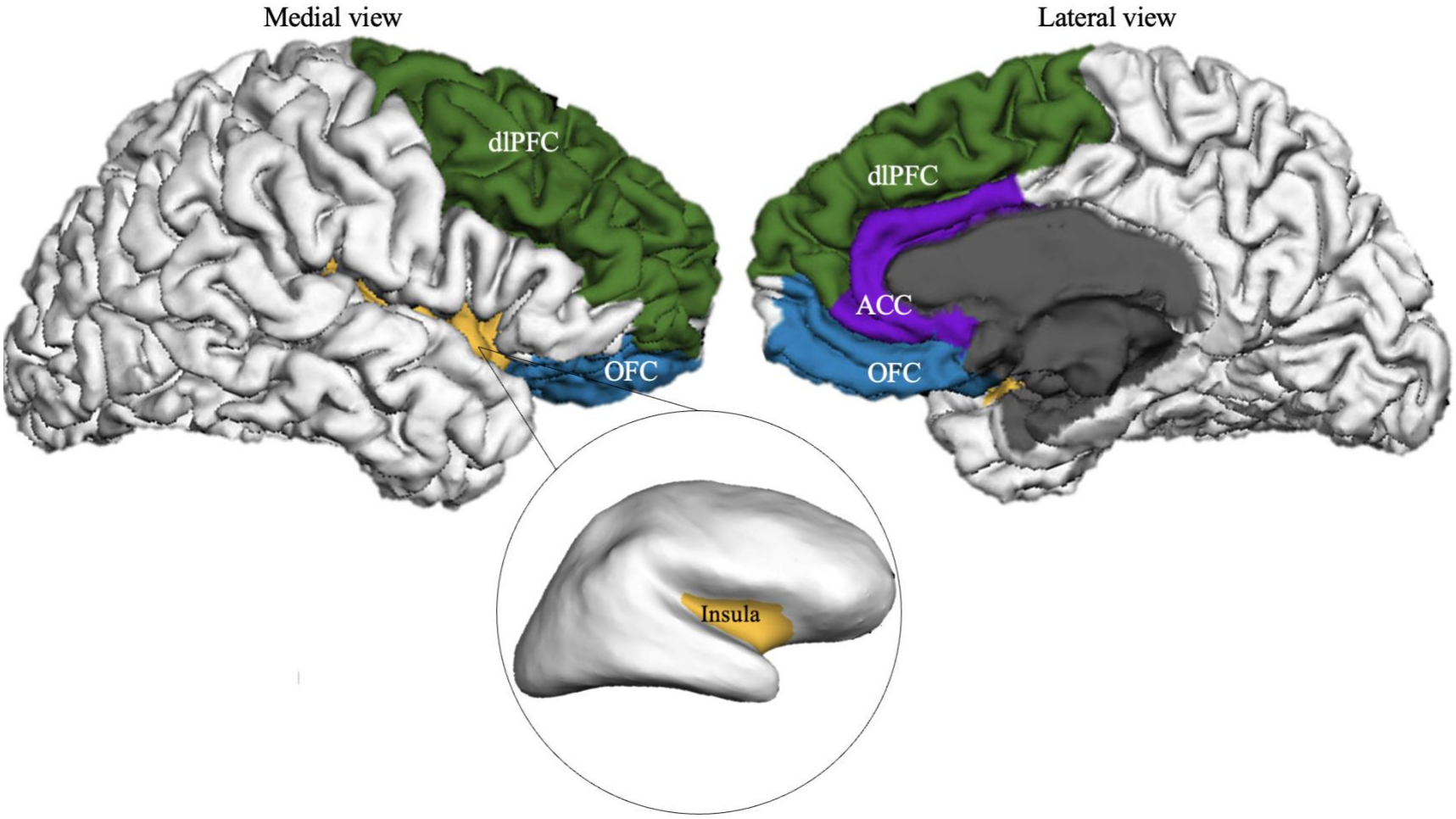
The cortex was parcellated into 34 regions bilaterally according to the Desikan-Killiany atlas (Desikan et al., 2006). Four regions of interest (ROI) were defined based on this atlas (*Figure 3. 1*). The resulting images were quality checked to ensure that all surfaces accurately followed the grey and white matter boundaries ensuring anatomical accuracy of surface detection and cortical parcellation. All images were retained for analysis following quality control. Reconstruction of the white and pial surfaces allowed for the calculation of cortical thickness, which was determined by measuring the shortest distance between grey/white matter boundaries and the pial surface at each surface point, these values were then averaged to obtain a single thickness value for each cortical region (Fischl & Dale, 2000). Our analysis was focused on thickness as a measure as it is highly heritable, while also more susceptible to environmental impact than volumetric or surface measures (Bootsman et al., 2017). Age, intercranial volume (ICV) and sex can confound analysis of cortical thickness, therefore these covariates were controlled for in statistical analysis (Barnes et al., 2010). Intercranial volume was calculated by dividing a predetermined constant with the factor by which the MR images are scaled to align to the MNI305 head atlas.

Reward related processes are underpinned by the interaction of network parts, however, only a limited number of the regions involved consistently display thickness or volumetric reductions in association with non-dependent alcohol use or bipolar disorder (see *Table 3.1)* (Koob & Volkow, 2016; Hibar et al., 2018). This suggests that only a subset of specific areas may be vulnerable to the combined impacts of alcohol use and BD. The regions which are persistently found to be impacted were defined bilaterally as the dlPFC, ACC,

OFC, and insula. The dlPFC was comprised of the superior frontal, rostral middle frontal and caudal middle frontal gyri. The ACC was identified by combining the caudal and rostral anterior cingulate, and the OFC was created by amalgamating the lateral and medial orbitofrontal cortices (*Figure 3.1*). Cortical thickness measurements were obtained for each ROI in each hemisphere, and were averaged across the dlPFC, ACC, and OFC, averaging was not required for the insula as it was comprised of one region, thus creating left and right measurements for each ROI. Two outliers in cortical thickness measurement, defined as more than 3 times the standard deviation above or below the mean were detected in the left ACC only. These images were again visually checked, the images were found to accurately follow the grey and white matter boundaries demonstrating anatomically relevant parcellation and were subsequently retained for statistical processing.

Figure 3.1

Parcellation of Regions of Interest based on the Desikan-Killiney Atlas



Note: ACC: anterior cingulate cortex; dlPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex.

3.3.8 Statistical Analyses

Group differences in demographic and clinical data were assessed using Chi-squared for categorical data (sex, hazardous and binge alcohol use) or *T*-tests for normally distributed continuous data (bilateral dlPFC, ACC, OFC, insula). The Mann-Whitney U was used with non-normally distributed continuous data (AUDIT-C, age at MRI, Hollingshead Socioeconomic Scale, Hamilton Depression Rating Scale, and Young Mania Rating Scale). Hierarchical multiple regression analyses were conducted to assess the relationship between AUDIT-C score and separately to assess the interaction between AUDIT-C and diagnosis, on ROI cortical thickness in all cases, controlling for age, sex, ICV, and diagnosis. A *post-hoc* analysis of covariance (ANCOVA) was undertaken to assess if medication (lithium or anti-psychotic use), socioeconomic status, or mood scores explained the relationships found. All *p*-vales reported are those following correction for multiple comparisons using the False Discovery Rate (FDR) method (Benjamin & Hochberg, 1995). Each hemisphere was considered a family for multiple comparisons correction, that is correction was undertaken for the left hemisphere and the right hemispheres independently of each other. A two-tailed α level of 0.05 was used for testing statistical significance. All statistical analyses were performed using SPSS software v.24 (IBM Corp., New York, USA).

3.4 Results

3.4.1 Demographic and Clinical Characteristics

Eighty-six individuals participated in this study, 40 participants with a diagnosis of BD (33 BD-I, 7 BD-II) and 46 healthy controls. The groups were matched for age and sex as demonstrated in *Table 3.2*. A significant difference was found between the groups for depressive symptoms on the HDRS (U=339.5, p<0.000), and the YMRS (U=678.5, p=0.016). Of the 40 BD participants, the majority were euthymic at the time of scanning (n=28, 70%). There was a difference in socioeconomic status between the groups with BD participants more likely to demonstrate a lower status in comparison to controls (U=583.5, p=0.004).

Table 3.2.

Clinical and Demographic Characteristics of the Sample

	Healthy controls *n=46*	*Bipolar participants* *n=40*	*Statistical Comparison* *Test statistic, p*
Sex (f:m, n)	30:16	21:19	χ^2=1.434, 0.231
Age at MRI (years)	40.98±14	43.08±13	U=858, 0.591
SES status (mean±SD)	42.2±16	31.52±16	U=583.5, 0.004*
HDRS (mean±SD)	1.04±1.7	6.78±6.6	U=339.5, 0.000*
YMRS (mean±SD)	0.72±1.5	1.80±2.6	U=678.5, 0.016*
Lithium use (no, %)	0, 0	26, 65	-
Antipsychotic use (no, %)	0, 0	13, 33	-

Note: Socioeconomic status was assessed using the Hollingshead Scale(Hollingshead, 2011). AUDIT- C: Alcohol Use Disorders Identification Test-Consumption; f: female; HDRS:

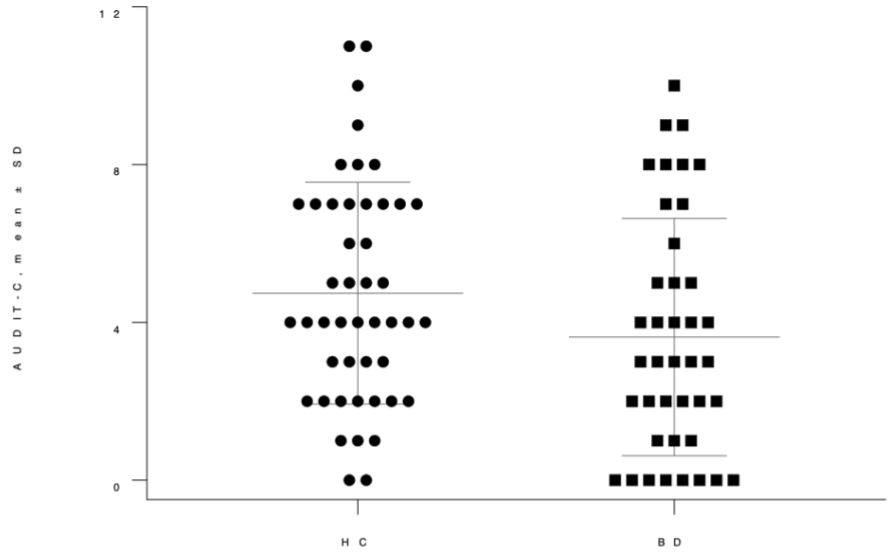
Hamilton Depression Rating Scale; m: male; YMRS: Young Mania Rating Scale. Data are presented as mean$\pm$sd. *significant at α=0.05.

3.4.2 Alcohol Use Scores

There was no difference between the diagnostic groups in alcohol use scores or for the potential to consume alcohol at harmful levels, defined as, scoring >5 on the AUDIT-C (χ^2=1.006, p=0.316) (*Figure 3.2).* Forty six percent of HC and 35% of BD participants reported scores that indicated a possibility for harmful alcohol use. Overall the diagnostic groups did not differ significantly in their frequency of binge drinking episodes, defined as consuming six or more alcoholic drinks in one sitting (Bush et al., 1998). The AUDIT-C results demonstrate a wide range of alcohol use within our cohort, ranging from abstinence (a score of 0) to a potential for consuming alcohol at binge levels daily or almost daily (our maximum score of 11).

Figure 3.2

No Difference in Alcohol Use Scores Between the Groups



	Healthy controls *n=46*	*Bipolar participants* *n=40*	*Statistical comparison* *Test statistic, p*
AUDIT-C (mean±SD)	4.74±2.81	3.63±3	U=713.5, *p*= 0.072
Positive for hazardous drinking (n,%)[a]	21 (46)	14 (35)	$\chi 2$=1.006, p= 0.316
Frequency of binge (n)[b]	Never: 15 Less than Monthly: 16 Monthly: 5 Weekly: 10	Never: 19 Less than Monthly: 11 Monthly: 5 Weekly: 5	$\chi 2$=2.658, *p*= 0.447

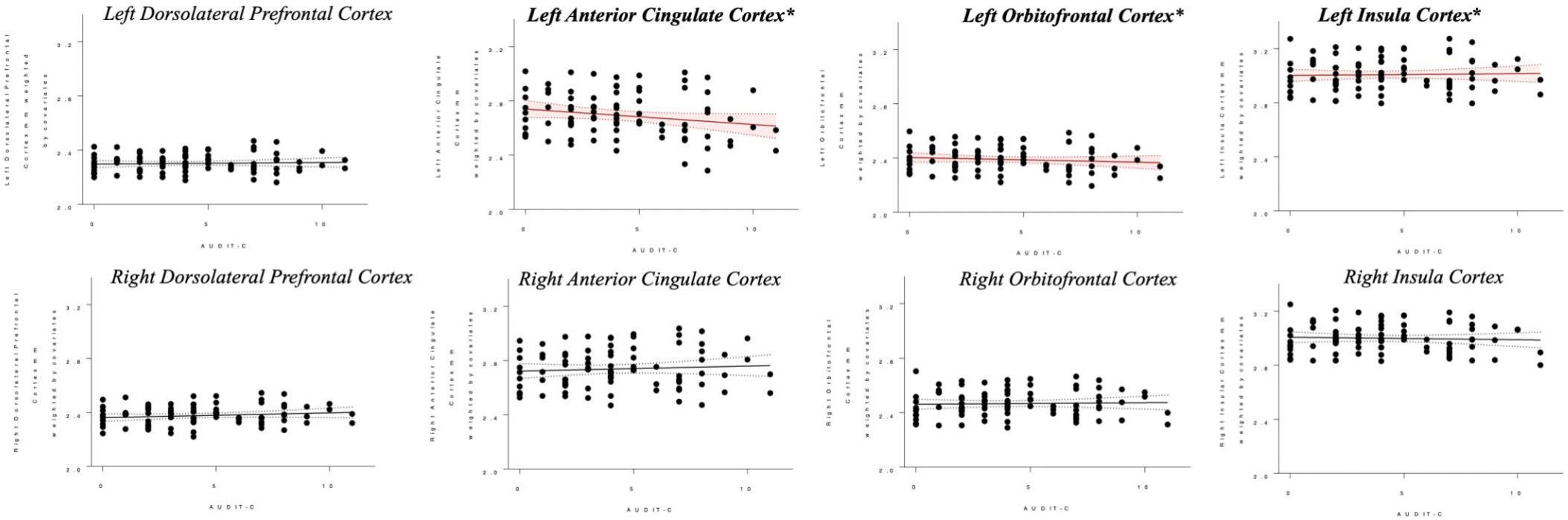
Note: [a]:Hazardous use is defined as scoring >5 in the AUDIT-C. [b]:A binge is defined as drinking more than 6 standard drinks in one setting.

3.4.3 Alcohol and Cortical Thickness

A multiple regression analysis, controlling for ICV, age, sex, and diagnosis demonstrated that alcohol use scores were associated with lowered cortical thickness in the left ACC, left OFC, and left insula areas for all participants (*Figure 3.3*; *Tables 3.3- 3.10*). The model demonstrated that for each one-point increase in AUDIT-C score, the left ACC is predicted to reduce by 0.027mm, the left OFC by 0.015mm, and the left insula by 0.015mm. These predicted alterations represent a change of 1% for left ACC, 0.63% for left OFC, and 0.5% for left insula as a proportion of the mean thickness of the sample. Tests to assess if the data met the assumption of collinearity indicated that multicollinearity was not a concern (Tolerance = 0.734 VIF = 1.362). There were no other significant associations between reward related structures or AUDIT-C score among the hypothesised cortical areas (*Figure 3.3; Table 3.3*).

Figure 3.3

Alcohol Use is Associated with Reductions of Cortical Thickness in Specific Reward Related Structures



Note: ACC: anterior cingulate cortex; dlPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex; ΔR^2: change in R^2,*significant at α<0.05. Regression analysis controlled for age, sex, ICV and diagnosis.

Table 3.3

Multiple Regression Examining the Effect of Alcohol on Left Dorsolateral Prefrontal Cortex Thickness

Left Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.004(0.001)	-0.445	-4.233	<0.001			
ICV	2.764E-8(0.00)	0.039	0.279	0.781			
Sex	-0.040(0.040)	-0.149	-1.011	0.315			
Diagnosis	-0.031(0.028)	-0.116	-1.011	0.274			
AUDIT-C	-0.007(0.005)	-0.164	-1.413	0.162	0.162	0.210	0.020

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.4

Multiple Regression Examining the Effect of Alcohol on Right Dorsolateral Prefrontal Cortical Thickness

Right Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.005(0.001)	-0.510	-5.018	<0.001			
ICV	1.162E-7(0.00)	0.156	1.155	0.252			
Sex	-0.026(0.041)	-0.092	-0.650	0.518			
Diagnosis	0.008(0.029)	0.030	0.295	0.769			
AUDIT-C	-0.005(0.005)	-0.113	-1.005	0.318	0.318	0.261	0.009

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.5

Multiple Regression Examining the Effect of Alcohol on Left Anterior Cingulate Cortical Thickness

Left Anterior Cingulate Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.011(0.002)	-0.549	-6.026	<0.001			
ICV	-3.153E-7(0.00)	-0.226	-1.870	0.065			
Sex	-0.106(0.068)	-0.198	-1.555	0.124			
Diagnosis	-0.106(0.048)	-0.202	-2.213	0.030			
AUDIT-C	-0.027(0.009)	-0.300	-2.984	0.004	0.016*	0.407	0.066

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.6

Multiple Regression Examining the Effect of Alcohol on Right Anterior Cingulate Cortical Thickness

Right Anterior Cingulate Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.010(0.001)	-0.645	-7.328	<0.001			
ICV	-1.686E-7(0.00)	-0.147	-1.259	0.212			
Sex	-0.063(0.054)	-0.143	-1.160	0.249			
Diagnosis	-0.025(0.038)	-0.059	-0.669	0.505			
AUDIT-C	-0.008(0.007)	-0.115	-1.187	0.239	0.318	0.446	0.010

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.7

Multiple Regression Examining the Effect of Alcohol on Left Orbitofrontal Cortical Thickness

Left Orbitofrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.007(0.001)	-0.560	-5.762	<0.001			
ICV	6.718E-9(0.00)	0.008	0.060	0.952			
Sex	-0.049(0.045)	-0.148	-1.087	0.281			
Diagnosis	-0.040(0.032)	-0.123	-1.261	0.211			
AUDIT-C	-0.015(0.006)	-0.269	-2.508	0.014	0.025*	0.324	0.053

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.8

Multiple Regression Examining the Effect of Alcohol on Right Orbitofrontal Cortical Thickness

Right Orbitofrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.007(0.001)	-0.630	-7.030	<0.001			
ICV	1.038E-8(0.00)	0.013	-0.108	0.914			
Sex	-0.085(0.039)	-0.276	-2.202	0.031			
Diagnosis	-0.033(0.027)	-0.107	-1.193	0.236			
AUDIT-C	-0.013(0.005)	-0.243	-2.460	0.016	0.054	0.426	0.043

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.9

Multiple Regression Examining the Effect of Alcohol on Left Insula Cortical Thickness

Left Insula Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.009(0.001)	-0.640	-7.161	<0.001			
ICV	9.445E-8(0.00)	0.097	0.809	0.421			
Sex	-0.056(0.047)	-0.151	-1.201	0.233			
Diagnosis	-0.055(0.033)	-0.151	-1.669	0.099			
AUDIT-C	-0.015(0.006)	-0.237	-2.385	0.019	0.025*	0.421	0.041

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

Table 3.10

Multiple Regression Examining the Effect of Alcohol on Right Insula Cortical Thickness

Right Insula Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.008(0.001)	-0.576	-6.001	<0.001			
ICV	-1.528E-8(0.00)	-0.016	-0.125	0.901			
Sex	-0.058(0.049)	-0.157	-1.171	0.245			
Diagnosis	-0.048(0.035)	-0.131	-1.364	0.176			
AUDIT-C	-0.015(0.007)	-0.239	-2.254	0.027	0.054	0.342	0.042

Note: ΔR^2 refers to the additional variance explained by the inclusion of the final variable. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$.

3.4.4 Bipolar Disorder and Cortical Thickness

Between group testing demonstrated that BD participants demonstrated similar values in the thickness of cortical regions compared to healthy controls (*Table 3.4).*

Table 3.11

No Difference in Cortical Thickness of Reward Related Structures between the Groups

	Healthy controls (Mean mm±SD)	*Bipolar participants (Mean mm±SD)*	*Statistical Comparison T, p*
Left dlPFC	2.32±0.14	2.29±0.12	0.948, 0.346
Right dlPFC	2.37±0.14	2.38±0.14	0.176, 0.861
Left ACC	2.73±0.29	2.64±0.22	1.485, 0.141
Right ACC	2.75±0.22	2.72±0.22	0.603, 0.548
Left OFC	2.41±0.18	2.37±0.15	0.905, 0.368
Right OFC	2.48±0.16	2.46±0.15	0.689, 0.493
Left Insula	3.03±0.19	2.98±0.17	1.251, 0.214
Right Insula	3.02±0.18	2.98±0.19	1.300, 0.197

Note: ACC: anterior cingulate cortex; dlPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex.

3.4.5 Alcohol, Bipolar Disorder and Cortical Thickness

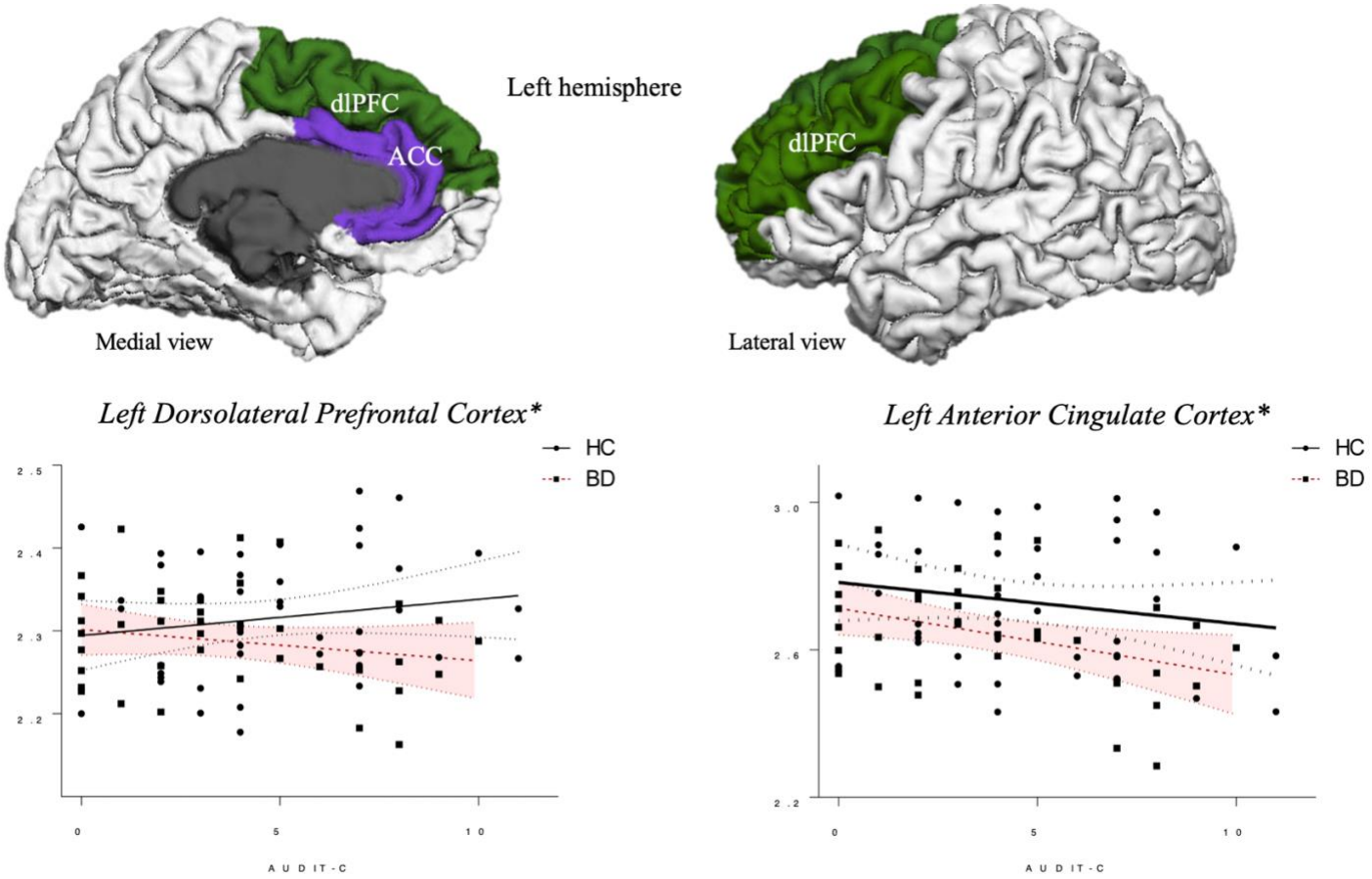
The interaction term between diagnosis and AUDIT-C (controlling for ICV, age and sex) was found to be significant predictor of lowered cortical thickness in the left dlPFC, and left ACC (*Figure 3.4*). Tests to assess if the data met the assumption of collinearity indicated that multicollinearity was not a concern (Tolerance = 0.254, VIF = 3.936). Visualisation of

the data demonstrates a complex relationship between alcohol use scores and diagnosis for the significant ROI (*Figure 3.4; Tables 3.12-3.19; Supplementary Tables 3.1-3.8*). For the left dlPFC, regression lines intersect, with the significant result being driven by the BD participants, the slope of the line in the BD group varies in direction from that observed in the control group. However, within the left ACC, both groups demonstrate a negative slope, with a more dramatic or pronounced effect found in the BD group. Group comparisons demonstrated lowered cortical thickness in association with alcohol use in the left dlPFC in BD (T=-2.237, p=0.032), but not in healthy controls (T=0.339, p=0.737), and of left ACC in BD (T=-3.187, p=0.003), but not in control participants (T=-0.914, p=0.366; *Supplementary figure 3.4)*.

Potential confounds to significant results related to medication and mood ratings for BD participants, and in SES scores for all participants were investigated. Lowered cortical thickness in the left dlPFC, and left ACC was compared between those taking and not taking lithium or antipsychotics in BD. Lithium use was not associated with the thickness of the left dlPFC (F(4,35)=0.035, p=0.852) or of the left ACC ($F(4, 35)$=0.057, p= 0.812) in BD participants (*Supplementary figure 3.5*). Additionally, there was no relationship between antipsychotic use or the thickness of the left dlPFC ($F(4,35)$=0.067, p=0.797), or the left ACC (F(4,35)=0.052, p=0.817; *Supplementary figure 3.6)*. Moreover, there was no association between alcohol use scores and mood rating scores using either the HDRS (r=-0.169, p=0.120) or the YMRS (r=0.059, p=0.587; *Supplementary figure 3.7)*. We found a difference in SES status between the two diagnostic groups, there was no association between SES status and thickness of the left dlPFC (r=-0.073, p=0.171) and the left ACC (r=-0.047, p=0.771).

Figure 3.4

Together Alcohol Use and Bipolar Disorder Predict Alterations in Specific Reward Related Structures



Note: ACC: anterior cingulate cortex; dlPFC: dorsolateral prefrontal cortex; *significant at α=0.05. Cortical measurements are in mm weighted by covariates. Regression analysis controlled for age, sex, diagnosis and ICV.

Table 3.12

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Left Dorsolateral Prefrontal Cortical Thickness

Left Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.005(0.001)	-0.502	-4.769	<0.001			
ICV	1.666-8(0.00)	0.023	0.173	0.863			
Sex	-0.056(0.039)	-0.208	-1.431	0.156			
Diagnosis	0.057(0.047)	0.215	-1.260	0.211			
AUDIT-C[a]	0.002(0.007)	0.041	0.284	0.777			
Dx*AUDIT-C[b]	-0.022(0.009)	-0.445	-2.320	0.023	0.046*	0.260	0.050

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.13

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Right Dorsolateral Prefrontal Cortical Thickness

Right Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.006(0.001)	-0.554	-5.370	<0.001			
ICV	1.074E-7(0.00)	0.144	1.081	0.283			
Sex	-0.039(0.041)	-0.138	-0.968	0.336			
Diagnosis[a]	0.079(0.048)	0.028	1.643	0.104			
AUDIT-C	0.002(0.007)	0.043	0.309	0.758			
Dx*AUDIT-C[b]	-0.018(0.010)	-0.340	-1.808	0.074	0.148	0.290	0.029

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.14

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Left Anterior Cingulate Cortical Thickness

Left Anterior Cingulate Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.012(0.002)	-0.599	-6.576	<0.001			
ICV	-3.343E-7(0.00)	-0.240	-2.036	0.045			
Sex	-0.134(0.067)	-0.251	-1.990	0.050			
Diagnosis[a]	-0.112(0.047)	0.090	0.589	0.018			
AUDIT-C	-0.011(0.011)	-0.120	-0.965	0.337			
Dx*AUDIT-C[b]	-0.038(0.016)	-0.392	-2.361	0.021	0.046*	0.446	0.039

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.15

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Right Anterior Cingulate Cortical Thickness

Right Anterior Cingulate Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.011(0.001)	-0.691	-7.814	<0.001			
ICV	-1.828E-7(0.00)	-0.159	-1.396	0.167			
Sex	-0.083(0.054)	-0.191	-1.560	0.123			
Diagnosis[a]	0.089(0.064)	0.207	1.398	0.421			
AUDIT-C	0.004(0.009)	0.049	0.404	0.687			
Dx*AUDIT-C[b]	-0.028(0.013)	-0.357	-2.214	0.030	0.120	0.478	0.032

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.16

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Left Orbitofrontal Cortical Thickness

Left Orbitofrontal Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.007(0.001)	-0.598	-6.036	<0.001			
ICV	-2.032E-9(0.00)	-0.002	-0.018	0.985			
Sex	-0.062(0.045)	-0.187	-1.365	0.176			
Diagnosis[a]	0.031(0.054)	0.094	0.567	0.572			
AUDIT-C	-0.007(0.007)	-0.135	-1.004	0.318			
Dx*AUDIT-C[b]	-0.017(0.011)	-0.292	-1.614	0.111	0.148	0.345	0.022

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.17

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Right Orbitofrontal Cortical Thickness

Right Orbitofrontal Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.007(0.001)	-0.635	-6.846	<0.001			
ICV	-1.139E-8(0.00)	-0.014	-0.118	0.907			
Sex	-0.087(0.040)	-0.281	-2.193	0.031			
Diagnosis[a]	-0.024(0.028)	-0.008	-1.197	0.606			
AUDIT-C	-0.012(0.007)	-0.227	-1.795	0.076			
Dx*AUDIT-C[b]	-0.002(0.009)	-0.036	-0.215	0.830	0.939	0.426	0.000

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.18

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Left Insula Cortical Thickness

Left Insula Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.009(0.001)	-0.675	-7.340	<0.001			
ICV	8.634E-8(0.00)	0.088	0.744	0.459			
Sex	-0.068(0.047)	-0.183	-1.441	0.154			
Diagnosis[a]	0.010(0.057)	0.027	0.176	0.861			
AUDIT-C	-0.008(0.008)	-0.127	-1.016	0.313			
Dx*AUDIT-C[b]	-0.016(0.011)	-0.239	-1.424	0.158	0.158	0.436	0.014

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 3.19

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Right Insula Cortical Thickness

Right Insula Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.008(0.001)	-0.574	-5.781	<0.001			
ICV	-1.481E-8(0.00)	-0.015	-0.120	0.905			
Sex	-0.057(0.050)	-0.155	-1.132	0.261			
Diagnosis[a]	-0.051(0.035)	-0.131	-1.349	0.181			
AUDIT-C	-0.015(0.008)	-0.245	-1.813	0.074			
Dx*AUDIT-C[b]	0.001(0.012)	0.014	0.076	0.939	0.939	0.342	0.000

Note: Variables used in these models have not been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

3.5 Discussion

Alcohol use is associated with lowered cortical thickness in the anterior cingulate, orbitofrontal, and insular cortices in a dose dependent manner that is not evident in the dorsolateral prefrontal cortex. Having a diagnosis of BD and using alcohol at non-dependent levels is associated with lowered cortical thickness in the dorsolateral and anterior cingulate cortices relative to those consuming alcohol who are psychiatrically healthy, despite a lack of effect in the dorsolateral cortex in association with alcohol use only. To the best of our knowledge, this is the first study to investigate the association between non-dependent alcohol use and structural alterations of distinct areas nested within reward circuitry in BD.

3.5.1 Alcohol Use in the Sample

There was no difference in alcohol use scores between the groups, demonstrating that for our sample, the amount of reported alcohol consumed did not differ between people with a diagnosis of BD as compared to healthy controls. Our data is limited as alcohol use is assessed using a self-report measure. Respondents often have inaccurate recall of their alcohol intake, differences between survey reporting and Department of Revenue figures in a recent Irish sample suggest that only 39% of alcohol intake is correctly reported (Long & Mongan, 2013). Should this be the case, our data may reflect an underestimation of our participant's alcohol use, however, the AUDIT-C has been demonstrated to reliably and sensitively identify non-dependent and hazardous alcohol use in a variety of settings (Dawson et al., 2005). Additionally, our alcohol-use scores are in line with those reported in a larger European study, suggesting that our figures may be an accurate reflection of alcohol use for all participants (Lange et al., 2016). Patients with BD report that they consume less

alcohol when in a euthymic period, and more in manic or depressed phases, our results are in the context of a euthymic mood state (Meyer et al., 2012).

3.5.2 Cortical Thickness in the Sample

We found that there was no difference in the thickness of cortical regions between the groups, this is surprising as structural alterations are a common feature of the disorder (Hibar et al., 2018; Hibar et al., 2016). However, effect sizes related to reductions in cortical thickness attributable to the disorder are found to be small in magnitude despite large sample sizes (Hibar et al., 2018). That we found no difference between the groups in cortical thickness is likely a reflection of our smaller sample size limiting our results in this instance. Cortical thickness reductions established in BD are assumed to reflect a reduction in the size and number of cells in a cortical column (Hibar et al., 2018; Rakic, 1988). Separately both alcohol use and BD are associated with reduced cortical thickness, which is posited as a marker for deterioration of neural tissue integrity including- neuronal apoptosis, a loss of dendritic spines, and oxidative stress (Crews, 2008; Manzo-Avalos & Saavedra-Molina, 2010; Vieta et al., 2018). The histology of cortical grey and white matter and scanner specific limitations can make accurate *in vivo* measurement of cortical thickness difficult (Fischl, 2012). However, implementing a surface deformation procedure to measure the MR intensity gradients at each point between the grey matter and cerebral spinal fluid has been found to reliably measure the thickness of grey matter in the human cortex (Fischl, 2012). Cortical thickness measurements using this method have been validated against histological measures, between scanner performance, and in studies comparing results between disease and healthy populations (Fischl, 2012). However, the resolution achieved in these studies, that of millimetres, is far greater than the size of a neuron therefore subtle effects of alcohol or diagnosis may be missed. Replication of these results undertaken in a larger sample,

alongside a preclinical study may provide richer information on subtle effects that cannot be detected at the resolution of a neuroimaging study.

3.5.3 Alcohol and Bipolar Disorder in Reward Related Structures

We have demonstrated that for participants with a diagnosis of BD, the consumption of alcohol at non-dependent levels is associated with lowered cortical thickness in the left dorsolateral and anterior cingulate cortices, in comparison to a control group of psychiatrically healthy participants. Our significant results display a complex relationship between alcohol use and specific reward related regions, with the right hemisphere failing to reach significance after correction for multiple comparisons. Our findings in relation to a differential effect in the BD group are discordant with the results of Lange *et al* (2016). The lack of an effect in the control group may be due to our sample size, alternatively our findings may point to specific regions related to reward and emotion processing which are vulnerable to compound environmental insult in the mood disorder. The dlPFC is responsible for cognitive control and the identification of reward, the region is found to be active during cue-induced alcohol craving (Beylergil et al., 2017), the ACC is involved in assessing risk, anticipating reward, and identifying emotionally relevant stimuli (Mashhoon et al., 2014). Hypoactivity of the left hemisphere and reduced cortical thickness in frontal lobes are frequently reported in BD, and are associated with emotional symptomatology and increased duration of illness (Bruder et al., 2017). Structural abnormalities within and between the dorsolateral and anterior cingulate cortices are suggested to relate to difficulties in emotional, attentional and cognitive control processing in BD (Jabbi et al., 2020). Moreover, the preservation of cortical thickness in the dlPFC is associated with a greater ability to maintain attention and control processes during a cognitive task (Burzynska et al., 2012). Reductions in cortical thickness in the dlPFC are found to predict the likelihood of increased alcohol use

and the increased frequency of binge alcohol use, suggesting a weakening of ability to control decisions governing alcohol use and an alteration of reward expectancies (Morris et al., 2019). Taken together, this suggests that structural alterations in association with alcohol use in the dorsolateral prefrontal cortex may lead to inefficiencies in the functioning of the reward network leading to a dysregulation of emotion processing. This may be pertinent for people with a diagnosis of BD where compromised cognitive control and changes in reward expectancies may increase the likelihood of continuing to consume alcohol despite negative health impacts and against the advice of treating clinicians. Lithium has been associated with neuroprotective effects on the anterior cingulate cortex suggesting that structural alterations could be corrected for.(Emsell & McDonald, 2009). We undertook *post-hoc* analyses to assess the relationship between lithium use and dorsolateral and anterior cingulate cortices in our sample and did not find an association between lithium use and cortical thinning (*Supplementary figure 2*). Moreover, there was no relationship between our mild range of mood scores and alcohol use in our sample (*Supplementary figure 3)*. Our results suggest that non-dependent alcohol use in people with a diagnosis of BD in a euthymic state may contribute to the emergence of cognitive inflexibility and emotional lability contributing to a conferred vulnerability to relapse in the disorder.

3.5.4 Alcohol Use and Reward Related Structures

Complex behaviour emerges due to the interaction of network parts, together the dorsolateral, anterior cingulate, orbitofrontal cortices, and insula function to generate a subjective urge to consume alcohol (Naqvi & Bechara, 2010; Everitt & Robbins, 2005). The efficient functioning of these prefrontal areas is suggested to maintain non-dependent alcohol use, due to less craving induced activation coupled with stronger cognitive control (Gordon, 2016). However, cognitive inflexibility arising due to structural alterations, may contribute to

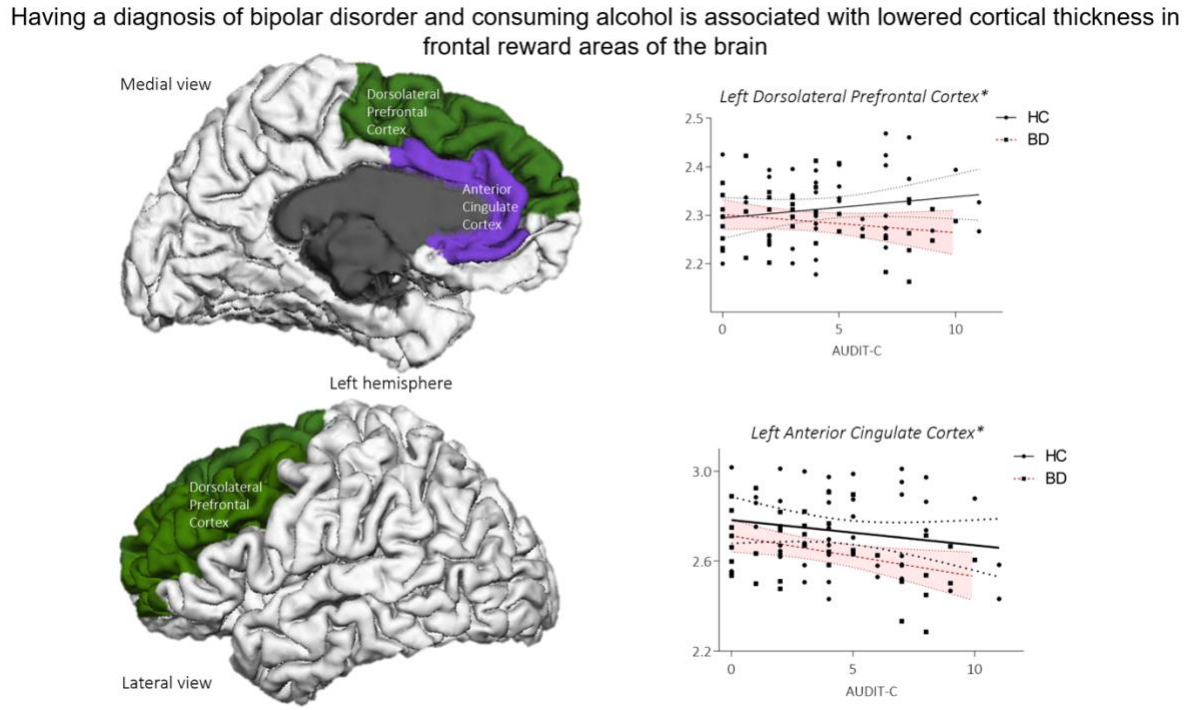
the continuing use of alcohol despite negative physical, social, and emotional impacts (Beylergil et al., 2017; Everitt & Robbins, 2005) We have demonstrated that non-dependent alcohol use was associated with lowered cortical thickness in the anterior cingulate, orbitofrontal, and insular cortices. Despite a hypothesised association between alcohol use and the dorsolateral prefrontal cortex, none was found. The OFC is involved in the control of flexible, goal directed behaviour, as well as the identification of reward. The region interacts bidirectionally with other areas involved in reward, motivation, and salience processes, with volumetric reduction of the orbitofrontal cortex associated with heavy drinking in emerging adulthood (Heikkinen et al., 2017; Moorman, 2018). The insula is responsible for the detection of salience of intrinsic and extrinsic events, and is positioned to initiate signals which increase the subjectively pleasurable feelings associated with alcohol use to motivate future use (Naqvi & Bechara, 2010; Menon & Uddin, 2010). Alterations in the functioning of these areas due to ongoing alcohol use, may lead to habitual alcohol consumption due to changes in salience perception and a dampening of the circuits ability to effectively inhibit impulsivity (Naqvi & Bechara, 2010; Adinoff, 2004). Our findings suggest that structural alterations within the anterior cingulate, orbitofrontal, and insular cortices may contribute to the functional alterations of this network. Within our study the right orbitofrontal cortex and insula failed to demonstrate significance following correction for multiple comparisons, as effect sizes are similar between the left and right hemispheres, we suggest that this is due to our sample size limiting our statistical power rather than a lateralised impact in these structures. The effect sizes we observed are moderate for the left anterior cingulate, and small for the left orbitofrontal and insular cortices, however, they appear in line with, if not slightly above what would be expected based on previous studies (*Table 1*). We demonstrate that at non-dependent levels of use, alcohol use is associated with reductions in cortical thickness in specific areas of the reward network.

3.5.5 Conclusion

We demonstrate an effect of alcohol use on specific areas of the reward network which may contribute to alterations in salience perception and control processes leading to an increased likelihood to consume alcohol. The structural findings specific to bipolar disorder are pertinent as they may contribute to compromised cognitive control and reward expectancies, leading to a dysregulation of emotion processing, and thus accelerating the possibility of relapse within the disorder. Further work should be undertaken to assess the network-wide effects of non-dependent alcohol use, the associations with symptomatology and the trajectory of mood lability and relapse in BD.

Supplementary Methods and Results Study One

Graphical Abstract



Widespread alterations in frontal, temporal and parietal cortical regions following non-dependent alcohol use and independently in bipolar disorder (BD) (Hibar et al., 2018; Lange et al., 2016). Individuals with bipolar disorder (BD) who engage in non-dependent levels of alcohol use have demonstrated a poor clinical trajectory, with increased numbers of depressive, and (hypo)manic episodes noted (Goldstein et al., 2006; Gordon-smith et al., 2020). Therefore, people with BD, who consume alcohol maybe increasing their risk of compound cortical alteration.

This manuscript aimed to identify if cortical thinning of specific consistently implicated structures within reward circuitry is associated with alcohol use, and if this effect is additively deleterious in BD.

3.6 Supplementary Methods

The Alcohol Use Disorder Identification Test (AUDIT) consumption sub-score (frequency and amount of alcohol consumed: AUDIT-C) was used in this analysis. The AUDIT-C comprises the first three questions of the AUDIT, and addresses current frequency of alcohol use, amount of drinks consumed, and frequency of binge drinking (≥6 standard drinks in one episode) (Bush et al., 1998). Each question is scored from 0 to 4, with a maximum possible score of 12. A standard drink in Ireland contains 10g of alcohol, within an Irish population a score of >5 on the AUDIT-C indicates a potential for harmful use, however this cut-off can vary country to country (Mongan & Long, 2015) (Figure XX).

Supplementary Figure 3.1

The Alcohol Use Disorder Identification Test Consumption (AUDIT-C)

ALCOHOL USE (AUDIT)

Have you drunk any alcohol in the last year? Yes ☐ No ☐

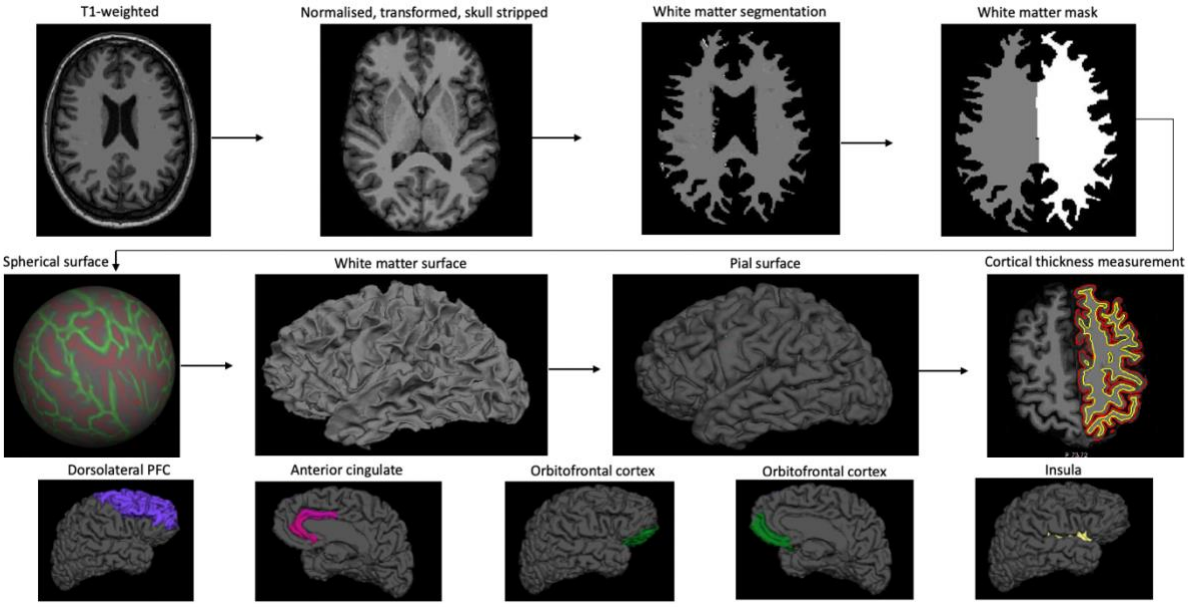
		0	1	2	3	4
1	How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week
2	How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more
3	How often do you have six or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily

3.6.1 Cortical Parcellation Procedure.

For cortical analysis, T1-weighted MR images were processed to correct for transformation to Talairach image space, motion distortions, intensity normalisation, and skull stripping (Dale et al., 1999). Images underwent quality control processes to ensure correct registration of image coordinates to Talairach coordinates, accurate correction for magnetic field inhomogeneity of T1-weighted images, and the satisfactory removal of non-brain tissue without the loss of brain tissue. The images then underwent segmentation of white matter based on image intensities, creating a WM volume, following this hemispheres are cut from each other and the brain stem removed creating a binary WM mask. The resulting white matter surface is deformed outwards to estimate the local intensity gradients between the grey matter and cerebral spinal fluid, which identifies the pial surface allowing for the detection of boundaries of grey, white and pial tissue in subject specific space (Fischl et al., 1999). The cortex was parcellated into 34 regions bilaterally according to the Desikan-Killiany atlas (Desikan et al., 2006). Reconstruction of the white and pial surfaces allowed for the calculation of cortical thickness, which was determined by measuring the shortest distance between grey/white matter boundaries and the pial surface at each surface point, these values were then averaged to obtain a single thickness value for each cortical region, this process was undertaken using Freesurfer (v5.3) (*Supplementary Figure 3.2*) (Fischl, 2012).

Supplementary Figure 3.2

Visualisation of Cortical Parcellation Procedure



Legend: PFC: prefrontal cortex

3.7 Supplementary Results

Two separate multiple regression analyses were undertaken to ascertain the association between alcohol use and a diagnosis of BD on cortical thickness, and independently alcohol use on cortical thickness controlling for diagnosis. Both regression analyses controlled for ICV, age and sex as these are known variables that can influence derived imaging measures (Barnes et al., 2010). Typical checks for distribution and outliers were undertaken, as well as multiple regressions using demeaned predictors to assess impact on the interaction model (Afshartous & Preston, 2011). It was found that there was no difference using demeaned values for AUDIT-C in the Interaction model for *p*, or R^2, change in R^2 values, there was a minor difference in beta and multicollinearity values. In both instances the tests for multicollinearity suggest that there is no serious issue of correlation between the variables in the model (Tolerance= 0.447, VIF= 2.238). The demeaned models are included in the supplemental regression reporting tables (*Supplementary Tables 3.1-3.8).*

It was decided at the time of manuscript preparation to report the main and interaction models without demeaned values as the variance in alcohol use scores and their association with cortical thickness was of primary interest. There was no difference found in significant results between the demeaned or original interactions.

The association between alcohol use scores and all regions of interest compared between groups is demonstrated in *Supplementary Figure 3.3.* We find that there is no association between alcohol use scores and lithium use in the regions which demonstrate cortical alterations in BD participants with increasing alcohol use (*Supplementary Figure 3.4*). Moreover, we find that there is no relationship between alcohol use and mood scores, or SES scores for all participants (*Supplementary Figures 3.5 and 3.6)*.

Supplementary Table 3.1

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Left Dorsolateral Prefrontal Cortex

Left Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.005(0.001)	-0.502	-4.769	<0.001			
ICV demeaned	1.666-8(0.00)	0.023	0.173	0.863			
Sex	-0.056(0.039)	-0.208	-1.431	0.156			
Diagnosis	-0.035(0.027)	-0.130	-1.260	0.211			
AUDIT-C demeaned	0.002(0.007)	0.041	0.284	0.777			
Dx*AUDIT-C demeaned	-0.022(0.009)	-0.336	-2.320	0.023	0.046*	0.260	0.050

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; * significant at $\alpha < 0.05$, [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.2

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Right Dorsolateral Prefrontal Cortex

Right Dorsolateral Prefrontal Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.006(0.001)	-0.554	-5.370	<0.001			
ICV demeaned	1.074E-7(0.00)	0.144	1.081	0.283			
Sex	-0.039(0.041)	-0.138	-0.968	0.336			
Diagnosis	0.006(0.028)	0.020	0.197	0.844			
AUDIT-C demeaned	0.002(0.007)	0.043	0.309	0.758			
Dx*AUDIT-C demeaned	-0.018(0.010)	-0.256	-1.808	0.074	0.148	0.290	0.029

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.3

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Left Anterior Cingulate Cortex

Left Anterior Cingulate Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.012(0.002)	-0.599	-6.576	<0.001			
ICV demeaned	-3.343E-7(0.00)	-0.240	-2.036	0.045			
Sex	-0.134(0.067)	-0.251	-1.990	0.050			
Diagnosis	-0.112(0.047)	-0.214	-2.405	0.018			
AUDIT-C demeaned	-0.011(0.011)	-0.120	-0.965	0.337			
Dx*AUDIT-C demeaned	-0.038(0.016)	-0.296	-2.361	0.021	0.046*	0.446	0.039

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.4

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Right Anterior Cingulate Cortex

Right Anterior Cingulate Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.011(0.001)	-0.691	-7.814	<0.001			
ICV demeaned	-1.828E-7(0.00)	-0.159	-1.396	0.167			
Sex	-0.083(0.054)	-0.191	-1.560	0.123			
Diagnosis	-0.030(0.037)	-0.070	-0.809	0.421			
AUDIT-C demeaned	0.004(0.009)	0.049	0.404	0.687			
Dx*AUDIT-C demeaned	-0.028(0.013)	-0.296	-2.214	0.030	0.120	0.478	0.032

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.5

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Left Orbitofrontal Cortex

Left Orbitofrontal Cortex

	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.007(0.001)	-0.598	-6.036	<0.001			
ICV demeaned	-2.032E-9(0.00)	-0.002	-0.018	0.985			
Sex	-0.062(0.045)	-0.187	-1.365	0.176			
Diagnosis	-0.043(0.031)	-0.132	-1.362	0.177			
AUDIT-C demeaned	-0.007(0.007)	-0.135	-1.004	0.318			
Dx*AUDIT-C demeaned	-0.017(0.011)	-0.220	-1.614	0.111	0.148	0.345	0.022

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.6

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Right Orbitofrontal Cortex

Right Orbitofrontal Cortex							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.007(0.001)	-0.635	-6.846	<0.001			
ICV demeaned	-1.139E-8(0.00)	-0.014	-0.118	0.907			
Sex	-0.087(0.040)	-0.281	-2.193	0.031			
Diagnosis	-0.033(0.028)	-0.108	-1.197	0.235			
AUDIT-C demeaned	-0.012(0.007)	-0.227	-1.795	0.076			
Dx*AUDIT-C demeaned	-0.002(0.009)	-0.027	-0.215	0.830	0.939	0.426	0.000

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.7

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Left Insula Cortex

Left Insula Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.009(0.001)	-0.675	-7.340	<0.001			
ICV demeaned	8.634E-8(0.00)	0.088	0.744	0.459			
Sex	-0.068(0.047)	-0.183	-1.441	0.154			
Diagnosis	-0.058(0.033)	-0.158	-1.757	0.083			
AUDIT-C demeaned	-0.008(0.008)	-0.127	-1.016	0.313			
Dx*AUDIT-C demeaned	-0.016(0.011)	-0.180	-1.424	0.158	0.195	0.436	0.014

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 3.8

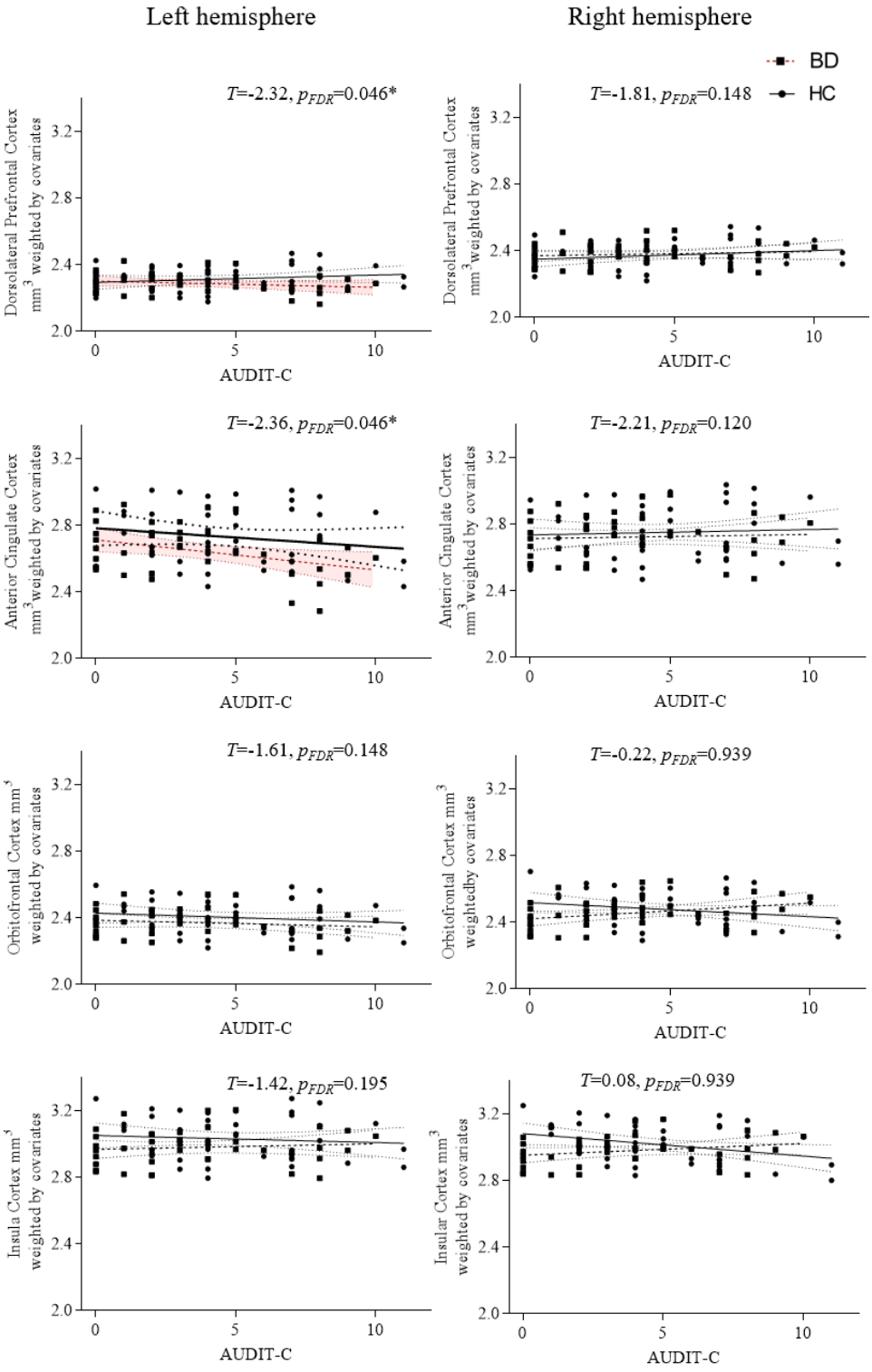
Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Cortical Thickness of Right Insula Cortex

Right Insula Cortex							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age demeaned	-0.008(0.001)	-0.574	-5.781	<0.001			
ICV demeaned	-1.481E-8(0.00)	-0.015	-0.120	0.905			
Sex	-0.057(0.050)	-0.155	-1.132	0.261			
Diagnosis	-0.047(0.035)	-0.131	-1.349	0.181			
AUDIT-C demeaned	-0.015(0.008)	-0.245	-1.813	0.074			
Dx*AUDIT-C demeaned	0.001(0.012)	0.010	0.076	0.939	0.939	0.342	0.000

Note: Variables used in these models have been demeaned. ICV: Intercranial Volume; SE: Standard Error; [a]: difference in beta, *T*, *p* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Figure 3.3

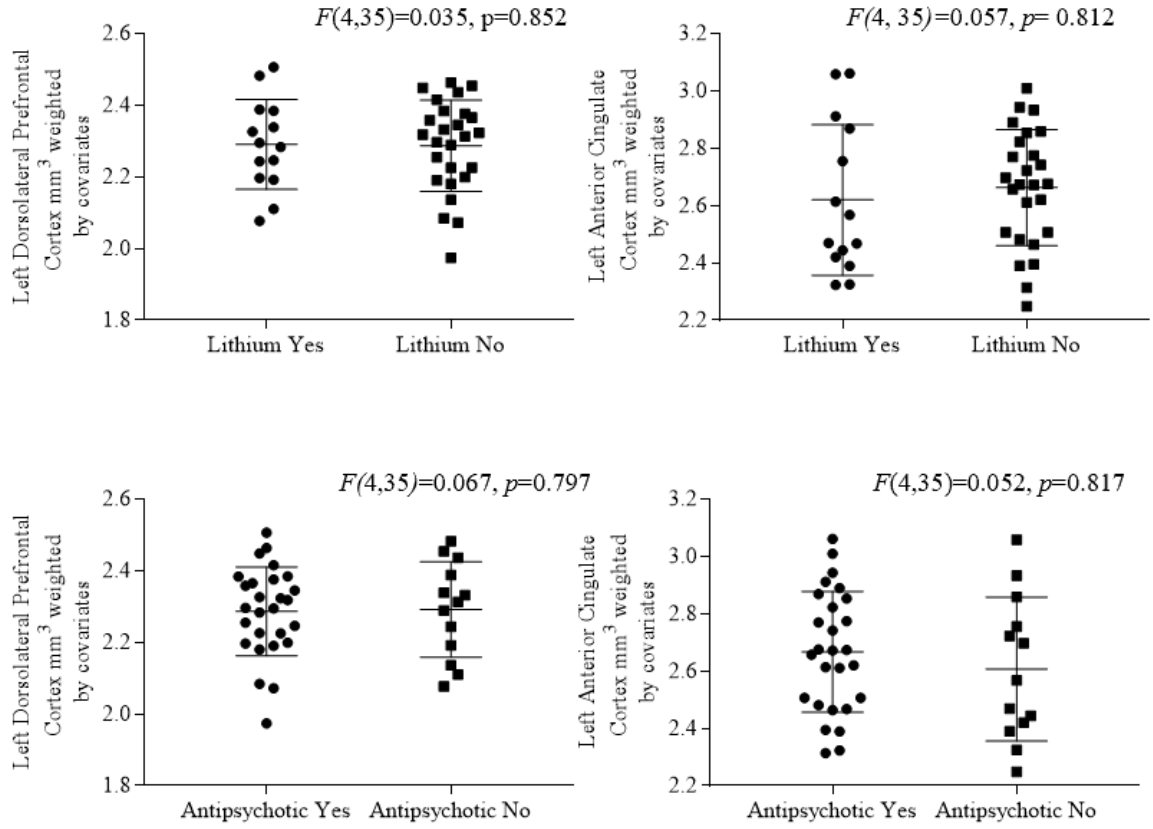
Associations Between Cortical Thickness and Alcohol Use Scores Compared Between Groups



Note: BD: Bipolar disorder; FDR: False Discovery Rate; HC: Healthy control.

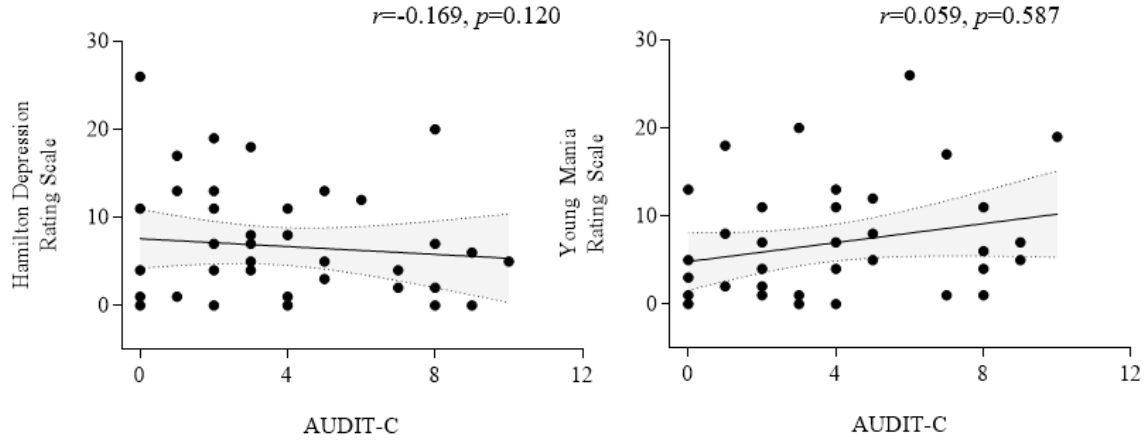
Supplementary Figure 3.4

Lithium Use is Not Associated with Cortical Thickness in Bipolar Disorder Participants



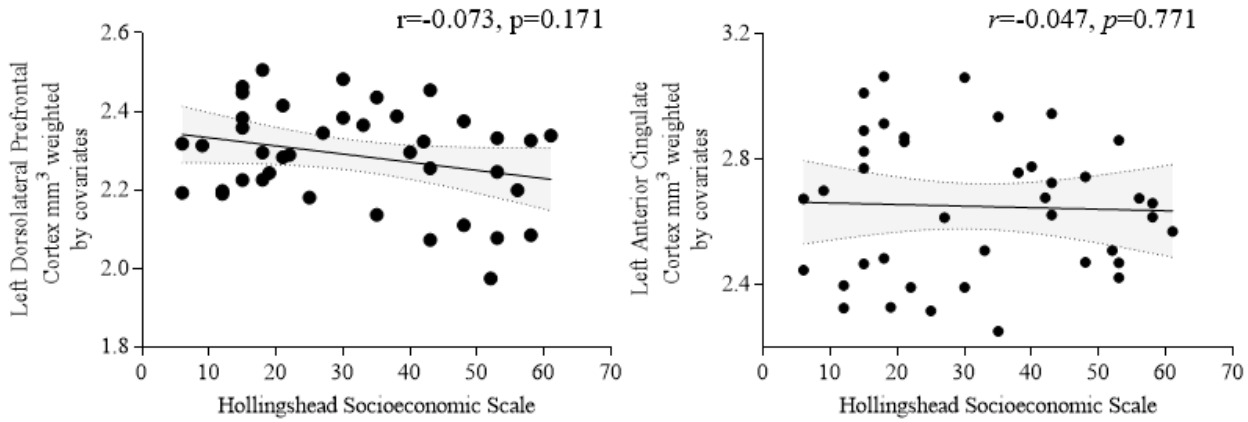
Supplementary Figure 3.5

Alcohol Use Scores Have No Relationship With Mood Rating Scores



Supplementary Figure 3.6

There is no Relationship Between Socioeconomic Status and Cortical Thickness



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Chapter Four

Study Two: Topological alteration is associated with non-dependent alcohol use in bipolar disorder

Author list: Fiona M. Martyn[1;2], Leila Nabulsi[1;3], Genevieve McPhilemy[1], Stefani O'Donoghue[1], Liam Kilmartin[4], Brian Hallahan[1], Colm McDonald[1], Dara M. Cannon[1].

Affiliations: [1]Centre for Neuroimaging, Cognition and Genomics (NICOG), Clinical Neuroimaging Laboratory, NCBES Galway Neuroscience Centre, College of Medicine, Nursing, and Health Sciences, National University of Ireland Galway, H91 TK33 Galway Ireland.

[2]School of Psychology, National University of Ireland Galway, Galway Ireland.

[3]Imaging Genetics Center, Mark and Mary Stevens Neuroimaging & Informatics Institute, University of Southern California, Marina del Rey, CA 90292, USA, Los Angeles, CA, United States.

[4]College of Engineering and Informatics, National University of Ireland Galway, Galway, Ireland.

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Authorship Confirmation Statement

FMM contributed to recruitment and data collection, performed MRI data quality checks, conducted all analyses and wrote the manuscript; DMC designed, obtained funding for and supervised data collection, analysis, and interpretation; BH and CMcD contributed to

recruitment and intellectual content; LN and GMP recruited and collected data, performed MRI data quality checks; SOD and LK contributed Matlab scripts.

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4.1 Abstract

Structural alterations in cortical thickness and the microstructural organisation of white matter are independently associated with non-dependent alcohol consumption and bipolar disorder(BD). Identifying their interactive and network level effects on brain topology may identify the impact of alcohol on reward and emotion circuitry, and its contribution to relapse in BD.

Thirty-four BD-I (DSM-IV-TR) and 38 psychiatrically healthy controls underwent T1 and diffusion-weighted MRI scanning, and the AUDIT-C to assess alcohol use. Connectomes comprised of 34 cortical and nine subcortical nodes bilaterally (Freesurfer v5.3) connected by fractional anisotropy-weighted edges derived from non-tensor based deterministic constrained spherical deconvolution tractography (ExploreDTI v4.8.6) underwent permutation-based topological analysis (NBS v1.2) and were examined for effects of alcohol use and diagnosis-by-alcohol use accounting for age, sex and diagnosis.

Alcohol was significantly related to a subnetwork, encompassing connections between fronto-limbic, basal ganglia and temporal nodes (F_{range}=5-8.4, p=0.031) and was not detected to have an effect on global brain integration or segregation. A portion of this network (18%), involving cortico-limbic and basal ganglia connections, was differentially impacted by alcohol in the BD relative to the control group (F_{range}=5-8.8, p=0.033), despite the groups' consuming similar amounts of alcohol (BD: mean±SD 4.95±3.0; HC 3.62±3.0, T=1.88, p=0.06).

Non-dependent alcohol use impacts brain architectural organization and connectivity within salience, reward, and affective circuitry. The relationship between alcohol use and topology of the network in BD suggests an interactive effect between specific biological vulnerability and alcohol use, which may explain susceptibility to increased risk of relapse in the disorder.

4.2 Introduction

Alcohol use is commonplace in society, 77% of our community consume alcohol, only 6.9% of this figure reach criteria for alcohol dependence (Department of Health, 2018). Alcohol use disorders and alcoholism are associated with negative impacts to the brain (Koob & Volkow, 2016), however, the topological impacts of non-dependent alcohol use on neuroanatomical organisation remains unknown. Structural alterations in cortical thickness and the microstructural organisation of white matter are described independently following alcohol consumption (Topiwala et al., 2017; Lange et al., 2016) and bipolar disorder (BD) (Favre et al., 2019; Hibar et al., 2018). Moreover, alcohol use in BD is associated with impaired clinical progress and increased depressive and (hypo)manic episodes suggesting a differential impact relative to psychiatrically healthy individuals (Gordon-smith et al., 2020). We move beyond traditional locationist approaches to investigate the interactive effects of alcohol use with a diagnosis of BD on brain network topology. This may elucidate relationships between alcohol use and relevant complex human behaviours such as reward seeking and emotion regulation. Using structural and diffusion-weighted magnetic resonance imaging (MRI), we aim to examine whether non-dependent alcohol consumption impacts brain architectural organisation, and if the effects are different in BD relative to a group of psychiatrically healthy controls.

Non-dependent alcohol use is associated with volumetric reduction of the hippocampus (Meda et al., 2019; Topiwala et al., 2017) and middle and inferior frontal gyri (Meda et al., 2017; Taki et al., 2006), and cortical thinning of medial and dorso-lateral prefrontal cortices as well as parieto-occipital areas (Morris et al., 2019; Lange et al., 2016) (*Table 1*). These structures have established roles within reward, and limbic circuitry, imbalance within these networks may result in difficulty regulating the rewarding experience

of alcohol thus impacting on emotional regulation (Koob & Volkow, 2016). College age participants who engage in binge alcohol use (>6 unit of alcohol in one sitting) demonstrate differential impacts to white matter tracts for men and women, a number of these tracts are associated with reward and emotion regulation processes (Smith et al., 2008). Moreover, alterations in white matter microstructural organisation have also been found within the corpus callosum in association with non-dependent alcohol use (Topiwala et al., 2017). Despite Topiwala *et al.* (2017) and Meda *et al.* (2019) demonstrating that increasing yet non-dependent alcohol consumption was associated with a higher risk of hippocampal atrophy, Naglich *et al.* (2018) found no relationship between drinks per week and hippocampal volume in a large sample. However, *post-hoc* investigations demonstrated that only participants who scored highly for depressive symptoms showed a reduction in hippocampal volume, suggesting that vulnerability toward mood dysregulation may interact with alcohol use. However, these studies do not provide information on the topology of the brain's network in association with non-dependent alcohol use: using a network neuroscience approach to guide this research can further our understanding on the interactive effects between brain and reward-related behaviour.

The utility of network analysis has been demonstrated in BD studies, where findings of structural dysconnectivity and reductions in cortical thickness have been expanded upon to encompass topological alterations associated with the disorder which can be related to complex symptomology (Ajilore et al., 2015). The network in BD demonstrates reductions in the global efficiency of information relay (Collin et al., 2016), as well as local inefficiency of information transfer between nodes belonging to the limbic network (Donoghue et al., 2017). At the mesoscale, a differentially connected subnetwork including structures involved in emotional regulation and reward, as well as divergent rich-club connectivity has been demonstrated (Nabulsi et al., 2019). Moreover, some of the variance in the structural

connectome is explained by cognitive deficits in participants with BD (Ajilore et al., 2015), alterations in intelligence measures are likely to be associated with selective changes in global network structure (McPhilemy et al., 2020). Changes in patterns of global, local, and mesoscale connectivity demonstrate how the interaction of topologies help us understand contributions to complex mental health diagnoses. Applying a network analysis to the brain topology in association with alcohol use, may elucidate interactive effects that impact on reward, or limbic circuitry as well as the observed increased vulnerability to relapse (Gordon-smith et al., 2020).

As a spatially embedded network the human brain achieves optimal functionality through a complex topological architecture characterised by small world properties, and multiscale subnetworks which can be decomposed into modules with few long-range connections between them (Bassett & Gazzaniga, 2011). The complexity of structural network topology confers an evolutionary advantage through robustness, and the capacity to respond to and act appropriately in rapidly changing environments (Bassett & Gazzaniga, 2011). Complex behaviour emerges through the patterning of subnetwork interactions, these networks appear at the mesoscale, between global and local connectivity, and can emerge, persist, and dissolve over topological scales (Bassett and Gazzaniga, 2011; Betzel and Bassett, 2017). Although it is possible to decompose these subnetworks into identifiable modules, the behavioural properties that arise as a function of the interactions within and between these communities are not similarly divisible (Bassett & Gazzaniga, 2011). This complex organisation gives rise to indirect interactions which allows for structural connectivity to shape but not define functional connectivity, these anatomical connections can therefore support a diverse range of functional network connections and a rich behavioural repertoire (Miŝic et al., 2016). Emergent behaviours are not only constrained by interactive networks of the brain, but also reflect interactions between the brain, body, and

environment which guide the ongoing process of diversity of network topology and behaviour (Sporns, 2018b).

Methodological advances in network neuroscience have allowed researchers to investigate topology using graph theory measures to describe the patterns of connectivity within the network (Bassett & Bullmore, 2017). These patterns allow the network to collectively transform inputs into signals relevant to internal and external stimuli which determine the behaviour of the organism (Suárez et al., 2020). Changes in network topology in association with environmental impacts such as alcohol use, or the biological influences of mood disorders such as BD, may identify disruptions to information segregation and integration across the network, thus increasing the cost of transition between brain states and potentially impacting behaviour (Shine & Poldrack, 2018). To date network-wide alterations in association with non-dependent alcohol use have not been investigated, with current research limited to changes in cortical thickness, volume, or alterations to the microstructural organisation of white matter (Topiwala et al., 2017; Lange et al., 2016). However, these studies are unable to provide descriptions of the patterns of relationships which underlie emergent behaviours, such as reward related activity and emotion regulation (Bassett et al., 2018). The use of alcohol can impact on behaviour through induction of feelings of pleasure and liking for alcohol and promote a desire to consume more alcohol while intoxicated (Gilman et al., 2008). Moreover, BD is also associated with heightened reward sensitivity and is associated with increased impulsivity contributing to emotional symptomatology within the disorder (Whitton et al., 2016). Therefore, understanding the network topology associated with a diagnosis of BD and non-dependent alcohol use may potentially contribute to our mechanistic understanding of the vulnerability to relapse in bipolar disorder.

The impact of non-dependent alcohol use on the topology of the network for all alcohol consumers and for people with a diagnosis of BD remains unknown. This research

will use the AUDIT-C: a tool validated to assess frequency and amount of alcohol use (Bush et al., 1998), structural T1-weighted and high angular resolution diffusion-weighted MRI, and a constrained spherical deconvolution (CSD) technique to recreate white matter trajectories to provide more reliable fibre reconstruction (Tournier et al., 2008). We aim to identify a subnetwork associated with alcohol use through the use of a permutation-based search of network connections, and to determine if there is a different relationship in those with BD, moreover we aim to investigate the effect of alcohol use overall on global network topology. We expect alcohol use will be associated with a subnetwork of the brain, involving reward and limbic nodes, which will be differently connected in those with BD in comparison to controls. Additionally, we expect that independently, alcohol use will be associated with alterations in global network connectivity.

Table 4.1.

Alterations to Grey and White Matter Associated with Alcohol Use or Bipolar Disorder

Authors	*Sample size (n)*	*Women: men*	*Age (mean years±SD)*	*MRI data analysis methods*	*Assessment of alcohol use*	*Findings (effect size)*
Moderate alcohol use studies						
Lange *et al,* 2016	609	48:52	34.2±9.9	Cortical thickness	AUDIT-C	↓ Parieto-occipital cortex (L: 4.6% R: 4% ΔR^2), ↓ Rostral middle frontal (L: 2.5% R: 1.6% ΔR^2), ↓ Superior frontal (L: 2.3%, R: 2.2% ΔR^2), ↓ Insula (R: 1.7% ΔR^2)
Morris, *et al,* 2019	706	51:49	28.8±3.6	Cortical thickness	SSAGA	↓ Left dlPFC (1.1% ΔR^2)
Taki *et al,* 2006	405	0:100	47±14.6	Grey matter volume	Lifetime alcohol history	↓ Middle frontal gyri (L: 17.3%; R: 15.3% R^2)
Topiwala *et al,* 2017	527	35:65	43±5.4	Grey matter volume, TBSS	CAGE, AUDIT	↓ Right hippocampus (16% R^2)
Naglich, *et al,* 2018	1848	59:41	49.8±10.5	Hippocampal volume	Drinks per week	↔ Hippocampal (L: 25.3%; R: 22.5% R^2)
Binge alcohol use studies						

Mashhoon, *et al*, 2014	54	48:52	22±1.2	Cortical thickness	AUDIT	↓ Right ACC (16.8% R^2)
Heikkinen *et al*, 2017	62	56:44	24.9±1.4	Grey matter volume	AUDIT-C	↓ Bilateral ACC (0.07), ↓ Right OFC (0.02), ↓ Right insula (0.06)∓
Meda *et al*, 2017	139	49:51	18.5	Grey matter volume	SSAGA, SCID	↓ Inferior frontal cortex ↓ Medial frontal cortex ↓ ACC ↓ Parahippocampus ↓Precentral gyrus ↓ Insula‡
Smith *et al*, 2015	40	50:50	20.48±1.77	Grey matter volume, TBSS	Alcohol use Questionnaire	↓ FA men ↑ FA women: Inferior fronto-occipital fasciculus, left corticospinal tract, corpus callosum: Prefrontal: 0.147/ sensory: 0.108/ parietal-temporal-occipital: 0.160 η^2.

Note: ACC: Anterior Cingulate Cortex; AUDIT-(C): Alcohol Use Disorders Identification Test (Consumption); dlPFC: Dorsolateral Prefrontal Cortex; L: Left; OFC: Orbitofrontal Cortex; R: Right; R^2: percentage of variance attributable to non-dependent alcohol use; SCID: Structured Clinical Interview DSM-V; SSAGA: Semi-structured assessment for the Genetics of Alcoholism; ΔR^2: change in R^2 or change in percentage of variance attributable to non-dependent alcohol use; ∓: effect size not defined in literature; ‡: effect sizes not given in literature; η^2: partial eta squared; ↓: reduction; ↔: no difference.

4.3 Methods

4.3.1 Participants

Participants are a subsample derived from a larger group identified in Nabulsi et al. (2019). All participants underwent a Structured Clinical Interview (SCID), patient or control edition, conducted by a registered psychiatrist. A diagnosis of BD (I or II) as determined by DSM-IV (American Psychiatric Association, 2000) criteria was confirmed for all BD participants. All participants were admitted to the study if they were aged 18-65 years, healthy controls (HC) had no diagnosis of any Axis-I disorder, no first degree relative with a diagnosed mental health condition, and no current or history of medication use for depression or anxiety. Exclusionary criteria for all participants were: a history of alcohol use disorder (AUD) or dependence, a loss of consciousness lasting more than five minutes, pregnancy or breastfeeding, any heart problems, or uncontrolled blood pressure. All participants gave written consent and ethical approval was granted by the Galway University Hospital Clinical Research Ethics Committee.

4.3.2 Assessment of Alcohol Use

The AUDIT-Consumption (AUDIT-C) is a validated scale arising from the first three questions of the larger 10 question AUDIT (Bush et al., 1998). The scale quantifies frequency and amount of alcohol use, as well as frequency of binge drinking episodes ($\geq$6 standard drinks in one episode). Each item is scaled from 0 to 4, with a maximum score of 12. According to Irish government drinking guidelines, a standard drink contains 10 grams of alcohol, within an Irish population a score of $>$5 on the AUDIT-C, indicates possible hazardous use (Long & Mongan, 2013). The AUDIT-C has been validated as a screening tool for a range of alcohol uses in a variety of settings (Kaarne et al., 2010).

4.3.3 Assessment of Severity of Signs and Symptoms

The Hamilton Depression Rating Scale *(*HDRS) is used to objectively and reliably quantify depressive episodes (Trajkovi et al., 2011; Hamilton, 1960). The questions are clinician rated, with a range of 0-53, a score of ≤8 indicates the absence of a depressive symptoms (Chengappa et al., 2003). The Young Mania Rating Scale (YMRS) has excellent reliability and validity indices for the identification of (hypo)manic symptoms (Young & Meyer, 1978). Scoring is based upon ratings during a clinical interview, a score of <7 indicates euthymia in the BD participants (Chengappa et al., 2003).

4.3.4 MRI Acquisition

All MRI scans were acquired using a high-resolution 3T Achieva scanner (Philips Medical Systems, Netherlands) with an 8-channel head coil. A structural T1-weighted *Magnetization Prepared Rapid Acquisition Gradient Echo* (MPRAGE) sequence was acquired. The parameters for this image acquisition were as follows: echo time (TE) 3.9 ms; repetition time (TR) 8.5 ms; flip angle 8°; slice thickness of 1 mm, 180 slices. *A diffusion-weighted scan was performed using high angular resolution (HARDI) with 61 diffusion directions. The acquisition parameters were: field of view (FOV): 198 × 259 × 125 mm, 66 slices, no gap, spatial resolution: 1.8 × 1.8 × 1.9 mm, matrix size: 144 x 144, TR/TE=514/59 ms, flip angle=90°, half k-space acquisition was used (half scan factor=0.681), SENSE parallel imaging factor=2.5, b =1200 s/mm^2, with SPIR fat suppression and dynamic stabilization in an image acquisition time of 17 minutes and 34.5 seconds.*

4.3.5 Construction of Network Matrices

Diffusion-weighted images were corrected for eddy current distortions, motion artefacts, and susceptibility effects using ExploreDTI (Leemans et al., 2009). All images were inspected for correct registration and quality, none were excluded based on quality inspection. White matter streamlines were reconstructed using deterministic nontensor-based constrained spherical deconvolution (CSD) (L_{max}=6) (Tournier et al., 2008). The RESTORE approach was used to perform diffusion eigenvector estimation (Chang et al., 2005). Fibre tracking began in each voxel and continued with a 1 mm step size and a 2 mm^3 seed point resolution, >30^o angle curvature threshold, 20-200 mm length and was terminated at a minimum FA of 0.2. The 'edges' of the connectivity matrices were defined as the recreated fibre tracts. T1-weighted images were processed to correct for motion, intensity normalisation, removal of non-brain tissue, and transformation to Talairach image space (Dale et al., 1999). Images were then segmented into grey and white matter through tessellation of the boundaries of grey and white matter and surface deformation in subject-specific space using Freesurfer (v5.3.0) (Fischl, 2012). All images were inspected for accurate segmentation and parcellation at the grey/white matter boundaries. The cortex was parcellated into 34 cortical and 9 subcortical brain regions bilaterally in subject-specific space based on the Desikan-Killiany atlas: these labels defined the nodes of the network matrices (Desikan et al., 2006). Edge and node information were combined to create binary and fractional anisotropy (FA) weighted connectivity matrices for each participant using ExploreDTI (Leemans et al., 2009).

4.3.6 Analysis of Subnetwork Connectivity

An analysis of a connected component associated with an effect of alcohol across all participants on FA-weighted networks, and for an interactive effect of alcohol and diagnosis was undertaken through permutation testing of graph connections while controlling for the

familywise error rate using Network Based Statistic (NBS) (Zalesky et al., 2010). A test statistic (regression model adjusting for age, sex and diagnosis) was computed to test for the extent and intensity of connectivity strength differences associated with AUDIT-C score, and an interaction between diagnosis and AUDIT-C score (M=5000, p=0.05), threshold of F>5 was used to obtain a set of suprathreshold connections (Zalesky et al., 2010). Images of the subnetwork were obtained using NeuroMArVL (http://immersive.erc.monash.edu.au/neuromarvl/).

4.3.7 Statistical Analyses

Group differences in demographic, clinical data, and graph theory measures were assessed using the Chi-squared test for categorical data (sex, education status, hazardous drinking, and frequency of binge drinking) or *T*-tests for normally distributed continuous data (AUDIT-C). Global and small world property measures derived from weighted and unweighted matrices included global efficiency (Eglobal), characteristic path length (CPL), density, clustering coefficient, strength, and small worldness were generated from connectivity matrices using Brain Connectivity Toolbox software (Rubinov & Sporns, 2010) accessed through MATLAB (v.R2015b; The MathWorks, 2015). The Mann-Whitney U was used with non-normally distributed continuous data (age at MRI, HDRS, YMRS and graph theory measures). Hierarchical multiple regression analyses were conducted to assess the relationship with AUDIT-C score and global graph metrics, controlling for age, sex, and diagnosis. An additional multiple regression was conducted with an interaction term between AUDIT-C and diagnosis. All *p*-vales reported were corrected for multiple comparisons using the False Discovery Rate (FDR) method (Benjamin & Hochberg, 1995). Correction was undertaken for all *p*-value results related to alcohol use, and then another family of

corrections for the *p*-value results related to the interaction between alcohol and diagnosis. Statistical analyses were performed using SPSS software (v.24; IBM Corp., USA).

4.4 Results

4.4.1 Sample Demographics and Clinical Characteristics

A total of 72 participants took part in this study, 38 healthy controls, and 34 BD (29 BD-I, 5 BD-II). There was no difference between the diagnostic groups with regard to age, sex or smoking status, there was however, a difference in socioeconomic status, with healthy controls more likely to report a higher status (*Table 4.2)*. There was a significant difference between the diagnostic groups for depressive symptoms on the HDRS (U=258, p<0.001). Of the 34 BD participants, the majority were euthymic at the time of scanning (n=23, 68%). There was no difference found for (hypo)manic symptoms assessed using the YMRS between the diagnostic groups

Table 4.2

Sample Demographics and Clinical Characteristics

	Healthy controls	*Bipolar participants*	*Statistical comparison*
	n=38	*n=34*	*Test statistic, p*
Sex (f/m: n)	24/14	18/16	χ^2=0.771, 0.380
Age at MRI (years)	39.3±14	42.8±13	*U*=560, 0.332
Smoker (yes/no)	7/31	8/25	χ^2=0.359, 0.549
SE status (mean±SD)	6.03±1.2	5.15±1.3	*U*=377, 0.002
HDRS (mean±SD)	1.1±1.8	6.56±6.9	*U*=258, <0.001
YMRS (mean±SD)	0.79±1.6	1.5±2.2	*U*=509.5, 0.073

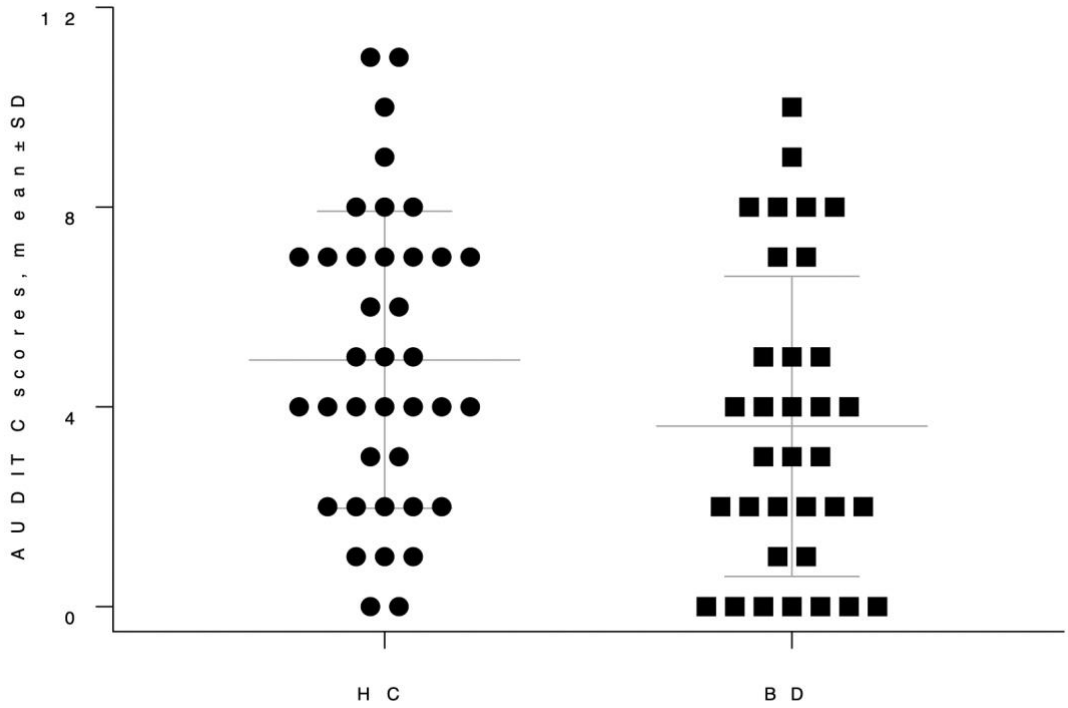
Note: f: female; HDRS: Hamilton Depression Rating Scale; m: male; Socio-economic status (SE) was assessed using the Hollingshead scale (Hollingshead, 2011): YMRS: Young Mania Rating Scale.

4.4.2 Comparable Alcohol Use Scores Between Healthy Controls and Bipolar Participants

There was no difference between the groups' AUDIT-C scores (*Figure 4.1; Table 4.3)* demonstrating that healthy controls and BD participants had comparable alcohol use scores. There was no difference in the likelihood of participants to consume alcohol at potentially hazardous amounts, scoring >5 (χ^2=2.299, p=0.129). The groups reported similar frequency of binge drinking episodes, defined as consuming six or more alcoholic drinks in one sitting.

Figure 4.1

Comparable Alcohol Use Scores Between Healthy Controls and Bipolar Participants



Note: AUDIT- C: Alcohol Use Disorders Identification Test- Consumption; BD: Bipolar participants; HC: Healthy controls; SD: standard deviation.

Table 4.3

No Difference in Alcohol Use Scores Between the Groups

	Healthy controls *n=38*	*Bipolar participants* *n=34*	*Statistical comparison between groups* *Test statistic, p*
AUDIT-C (mean±SD)	4.95±3.0	3.62±3.0	T= 1.884 p=0.064
Positive for hazardous drinking[a] *(n,%)*	19 (50)	11 (32)	χ^2 =2.299, p=0.129
Frequency of binge[b] *(n)*	Never: 11 Less than Monthly: 13 Monthly: 5 Weekly: 9	Never: 16 Less than Monthly: 10 Monthly: 4 Weekly: 4	χ^2 =3.139 p=0.371

Note: [a]Hazardous drinking is defined as scoring >5 in the AUDIT-C. [b]A binge is defined as drinking more than 6 standard drinks in one setting.

AUDIT- C: Alcohol Use Disorders Identification Test- Consumption;; SD: standard deviation.

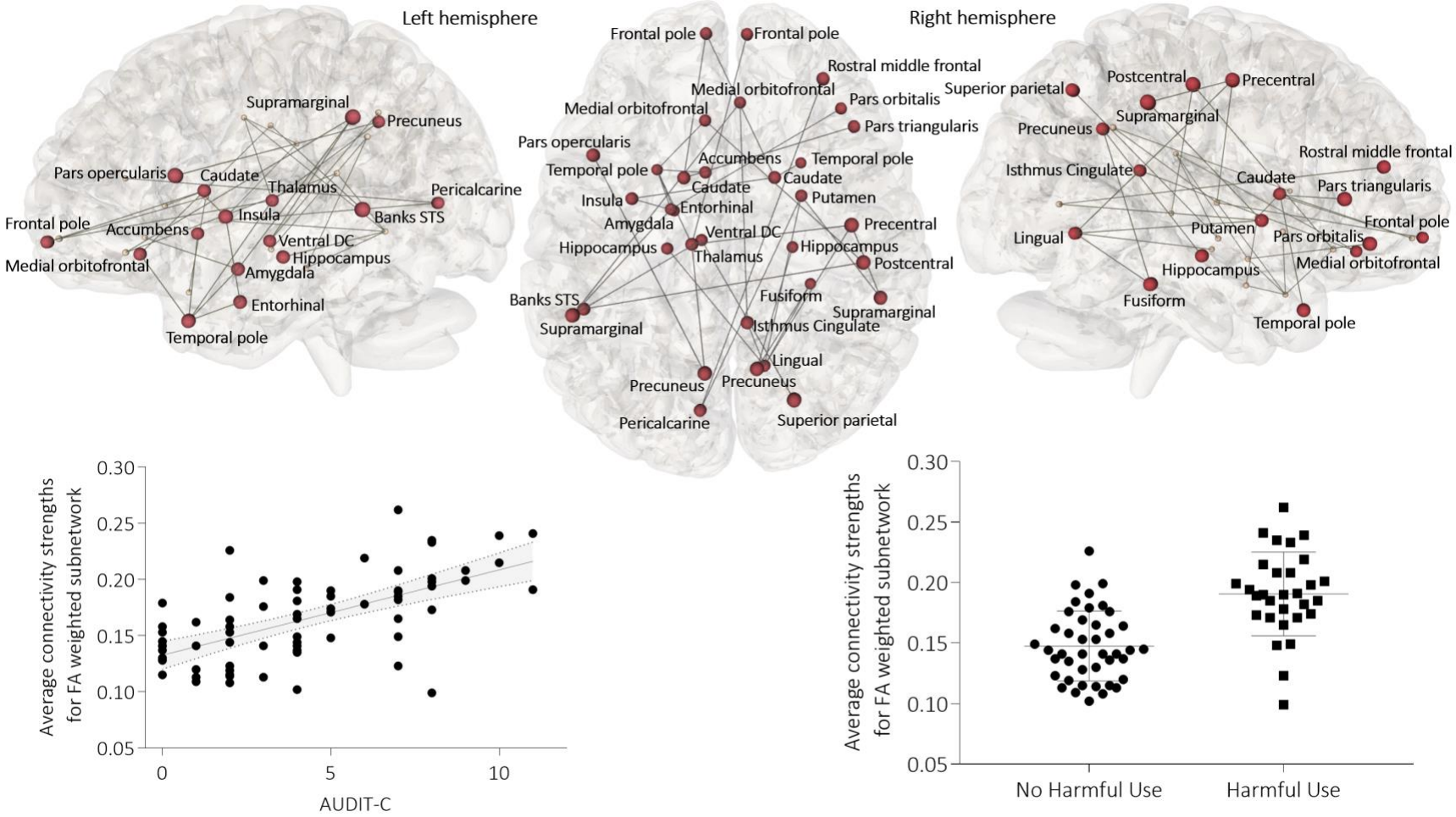
4.4.2 Alcohol Use and Subnetwork Analysis

Edge-level analysis revealed a subnetwork of the brain associated with alcohol use that was significant at threshold F_{range} 5-8.4, p=0.031 (*Supplementary Table 4.1* and *Figure 4.2*). This network remained significant when examined using extent or intensity, demonstrating that alcohol has a strong and distributed impact. Average connectivity strengths plotted against AUDIT-C score demonstrated that alcohol use was associated with an increase in connectivity strength within this network. Nodes involved in fronto-limbic, basal ganglia and temporal circuitry were involved in this connected component, in particular the nucleus accumbens, caudate, putamen, amygdala, hippocampus, temporal pole, lateral and medial frontal areas.

Further permutation analysis revealed that alcohol use is associated with a subnetwork involving cortico-limbic, and basal ganglia connections, with differential connectivity strengths in BD participants relative to controls, the subnetwork was significant at threshold F_{range} 5-8.8, p=0.033 (*Supplementary Table 4.2* and Figure *4.3*). This subnetwork included nodes involved in reward and emotion processes including caudate, hippocampus, anterior cingulate and orbitofrontal cortices, and superior and middle temporal gyri (*Figure 4.3)*. The interaction appears to be driven by the BD groups as increasing alcohol use scores were associated with lesser connectivity strengths for BD participants, this relationship is absent in the healthy control group.

Figure 4.2.

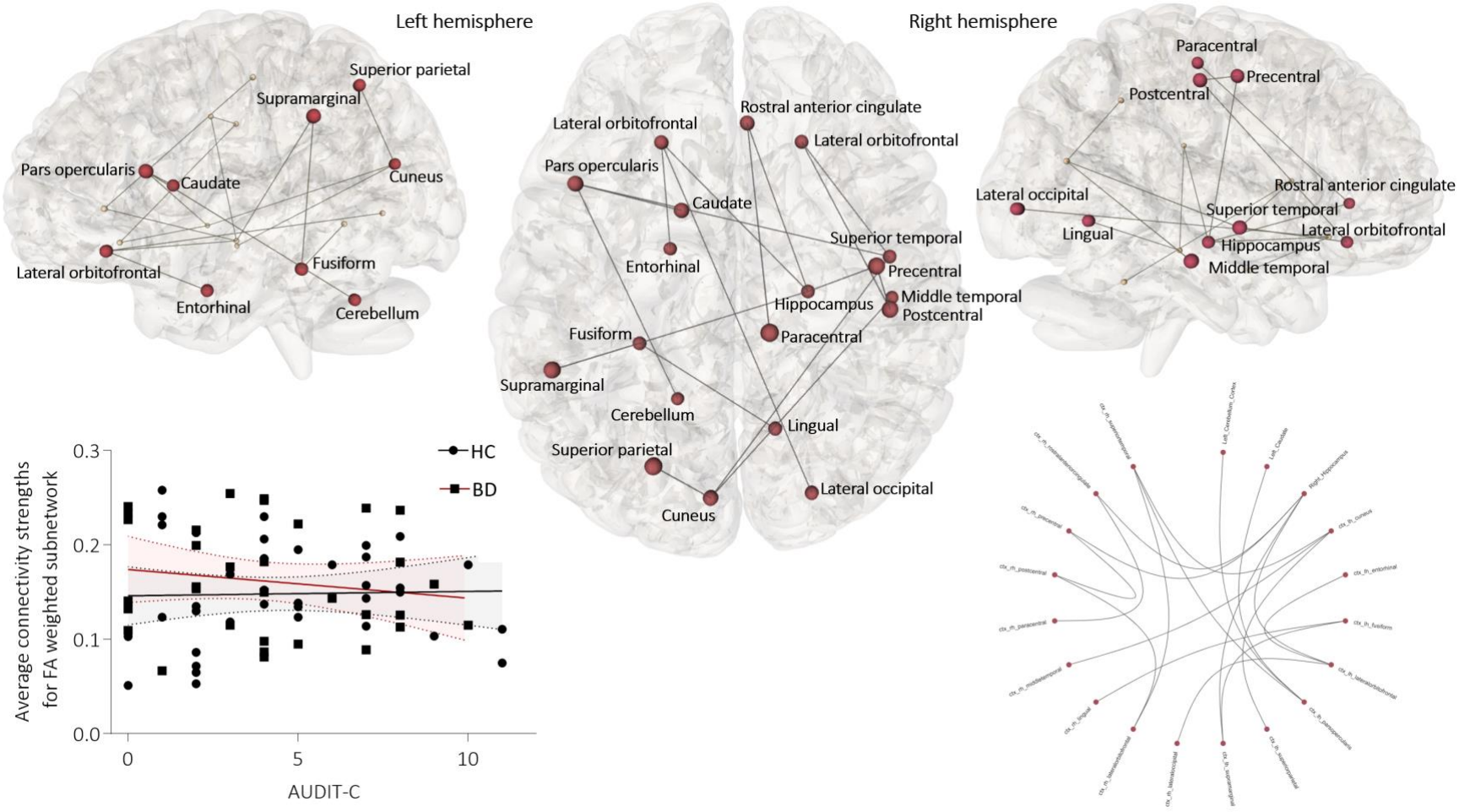
Alcohol Use Relates Positively with Connectivity in a Fronto-limbic, Basal Ganglia, and Temporal Subnetwork



Note: Subnetwork of the brain associated with AUDIT-C scores for all participants (F=8.4, p=0.031).

Figure 4.3.

In Bipolar Participants Relative to Controls, Connectivity Negatively Relates to Alcohol Use in a Cortico-limbic, Basal Ganglia Subnetwork



Note: Subnetwork of the brain associated with an interaction between diagnosis and AUDIT-C score (F=8.8, *p*=0.033)

4.4.4 Alcohol Use and Global Network Topology

Global topological organisation was not associated with alcohol use, which was reflected in a lack of alteration in information integration and segregation measures. There was no main effect of alcohol detected for any measure of the overall integration or segregation across the whole brain following correction for multiple comparisons (*Table 4.4-4.13;).* Tests to assess if the data met the assumption of collinearity indicated that multicollinearity was not a concern (Tolerance = 0.724, VIF = 1.381). Similarly, a diagnosis of BD and non-dependent alcohol use, did not appear to interact at the global brain level to impact overall pathlength, clustering or efficacy (*Table 4.14-4.23).* Testing to assess if the data met the assumption of collinearity again indicated that multicollinearity was not a concern (Tolerance = 0.255, VIF = 3.914).

Table 4.4

Multiple Regression Examining the Effect of Alcohol on $Eglobal_{Binary}$

Eglobal$_{Binary}$								
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	2.534E-5(0.000)	0.013	0.116	<0.001				
Sex	0.014(0.007)	0.266	2.140	0.036				
Diagnosis	-0.015(0.006)	-0.278	-2.419	0.018				
AUDIT-C	0.002(0.001)	0.267	2.086	0.041	0.278	0.204	0.052	0.065

Note: $_{Binary}$: referring to presence or absence of connection; Eglobal: Global efficiency; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.5

Multiple Regression Examining the Effect of Alcohol on CPL_{Binary}

CPL_{Binary}								
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	0.000(000)	-0.040	-0.345	<0.001				
Sex	0.002(0.010)	0.021	0.160	0.873				
Diagnosis	0.031(0.009)	0.394	3.317	0.001				
AUDIT-C	-5.067E-5(0.002)	-0.004	-0.029	0.977	0.977	0.152	0.000	0.000

Note: Binary: referring to presence or absence of connection; CPL: Characteristic path length; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.6

Multiple Regression Examining the Effect of Alcohol on $Density_{Binary}$

$Density_{Binary}$								
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	1.426E-5(0.000)	0.007	0.061	0.951				
Sex	0.007(0.007)	0.118	0.934	0.354				
Diagnosis	-0.021(0.007)	-0.366	-3.130	0.003				
AUDIT-C	0.001(0.001)	0.116	0.888	0.378	0.977	0.176	0.010	0.011

Note: $_{Binary}$: referring to presence or absence of connection; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.7

Multiple Regression Examining the Effect of Alcohol on Clustering Coefficient

Clustering Coefficient								
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-6.628E-5(0.000)	-0.054	-0.460	0.647				
Sex	-0.002(0.005)	-0.052	-0.395	0.694				
Diagnosis	-0.012(0.004)	-0.339	-2.805	0.007				
AUDIT-C	-2.860E-5(0.001)	-0.005	-0.038	0.970	0.970	0.121	0.000	0.000

Note: SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.8

Multiple Regression Examining the Effect of Alcohol on $CC_{Normalised}$ (γ)

$CC_{Normalised}$ (γ)								
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-0.001 (0.001)	-0.238	-2.051	0.044				
Sex	0.015(0.022)	0.087	0.672	0.504				
Diagnosis	0.054(0.020)	0.322	2.690	0.009				
AUDIT-C	0.001(0.004)	0.035	0.262	0.794	0.977	0.138	0.001	0.001

Note: $CC_{Normalised}$: $\frac{CC_{Global}}{CC_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.9

Multiple Regression Examining the Effect of Alcohol on $CPL_{Normalised}$ (λ)

$CPL_{Normalised}$ (λ)								
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-8.385E-5(0.000)	-0.128	-1.131	0.262				
Sex	-0.001(0.002)	-0.045	-0.358	0.721				
Diagnosis	0.007(0.002)	0.412	3.528	<0.001				
AUDIT-C	-7.092E-5(0.004)	-0.024	-0.183	0.855	0.977	0.181	0.000	0.000

Note: $CPL_{Normalised}$: $\frac{CPL_{Binary}}{CPL_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.10

Multiple Regression Examining the Effect of Alcohol on Small Worldness (σ)

Small Worldness (σ)								
	Beta(SE)	β	T	$P_{\text{Uncorrected}}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-0.001(0.001)	-0.249	-2.141	0.036				
Sex	0.016(0.019)	0.109	0.839	0.404				
Diagnosis	0.044(0.017)	0.304	2.535	0.014				
AUDIT-C	0.001(0.003)	0.033	0.250	0.803	0.977	0.135	0.001	0.001

Note: Small World: $\frac{CPl_{Normalised}}{CC_{Normalised}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.11

Multiple Regression Examining the Effect of Alcohol on $Eglobal_{FA}$

$Eglobal_{FA}$								
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-8.283E-5(0.000)	-0.086	-0.753	0.454				
Sex	0.000(0.003)	-0.013	-0.099	0.921				
Diagnosis	-0.009(0.003)	-0.337	-2.843	0.006				
AUDIT-C	0.000(0.001)	0.091	0.685	0.496	0.744	0.154	0.006	0.007

Note: Eglobal: Global efficiency; $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.12

Multiple Regression Examining the Effect of Alcohol on CPL_{FA}

CPL_{FA}								
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	0.002(0.002)	0.102	0.889	0.377				
Sex	0.119(0.056)	0.270	2.100	0.040				
Diagnosis	0.145 (0.052)	0.334	2.802	0.007				
AUDIT-C	0.010(0.009)	0.143	1.080	0.284	0.744	0.147	0.015	0.018

Note: CPL: Characteristic path length; $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.13

Multiple Regression Examining the Effect of Alcohol on $Strength_{FA}$

$Strength_{FA}$								
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2	f^2
Age	-0.003(0.008)	-0.038	-0.332	0.741				
Sex	-0.084(0.237)	-0.045	-0.354	0.724				
Diagnosis	-0.725 (0.217)	-0.393	-3.345	0.001				
AUDIT-C	0.013(0.040)	0.042	0.319	0.751	0.751	0.169	0.001	0.001

Note: FA: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.14

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Eglobal_{Binary}$

$Eglobal_{Binary}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	5.858E-5(0.000)	0.030	0.257	0.798			
Sex	0.015(0.007)	0.283	2.193	0.032			
Diagnosis[a]	-0.020(0.011)	-0.366	-1.813	0.074			
AUDIT-C	0.002(0.001)	0.218	1.366	0.177			
Dx*AUDIT-C[b]	0.001(0.002)	0.115	0.529	0.599	0.985	0.208	0.003

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $_{Binary}$: referring to presence or absence of connection; Eglobal: Global efficiency; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.15

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on CPL_{Binary}

CPL_{Binary}							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-6.871E-5 (0.000)	-0.024	-0.197	0.844			
Sex	0.003(0.011)	0.037	0.276	0.783			
Diagnosis[a]	0.025(0.017)	0.312	1.500	0.138			
AUDIT-C	-0.001(0.002)	-0.050	-0.305	0.761			
Dx*AUDIT-C[b]	0.002(0.003)	0.107	0.478	0.634	0.985	0.155	0.003

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Binary: referring to presence or absence of connection; CPL: Characteristic path length; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.16

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Density_{Binary}$

$Density_{Binary}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	1.590E-5(0.000)	0.008	0.065	0.948			
Sex	0.007(0.008)	0.119	0.902	0.370			
Diagnosis[a]	-0.021(0.012)	-0.370	-1.801	0.076			
AUDIT-C	0.001(0.002)	0.113	0.698	0.488			
Dx*AUDIT-C[b]	5.570E-5(0.002)	0.005	0.024	0.981	0.985	0.176	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $_{Binary}$: referring to presence or absence of connection; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.17

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Clustering Coefficient

Clustering Coefficient							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-5.928E-5(0.000)	-0.048	-0.390	0.698			
Sex	-0.002(0.005)	-0.045	-0.333	0.740			
Diagnosis[b]	-0.013(0.007)	-0.370	-1.744	0.086			
AUDIT-C	0.000(0.001)	-0.023	-0.136	0.892			
Dx*AUDIT-C[b]	0.00(0.001)	0.041	0.179	0.858	0.985	0.121	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.18

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $CC_{Normalised}$ (γ)

$CC_{Normalised}$ (γ)							
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.001(0.001)	-0.237	-1.951	0.055			
Sex	0.015(0.023)	0.088	0.650	0.518			
Diagnosis[a]	0.053(0.035)	0.319	1.515	0.134			
AUDIT-C	0.001(0.005)	0.033	0.199	0.843			
Dx*AUDIT-C[b]	0.000(0.007)	0.004	0.019	0.985	0.985	0.138	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $CC_{Normalised}$: $\frac{CC_{Global}}{CC_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.19

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $CPL_{Normalised}$ (λ)

$CPL_{Normalised}$ (λ)							
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-8.495E--5(0.000)	-0.129	-1.093	0.278			
Sex	-0.001(0.002)	-0.047	-0.357	0.722			
Diagnosis[a]	0.008(0.004)	0.420	2.050	0.044			
AUDIT-C	-5.630E-5(0.000)	-0.019	-0.117	0.907			
Dx*AUDIT-C[b]	-3.752E-5(0.001)	-0.011	-0.051	0.959	0.985	0.181	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $CPL_{Normalised}$: $\frac{CPL_{Binary}}{CPL_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.20

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Small Worldness (σ)

Small Worldness (σ)							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.001(0.001)	-0.251	-2.065	0.043			
Sex	0.016(0.020)	0.106	0.784	0.436			
Diagnosis[a]	0.046(0.031)	0.318	1.510	0.136			
AUDIT-C	0.001(0.004)	0.041	0.249	0.804			
Dx*AUDIT-C[b]	0.000(0.006)	-0.019	-0.082	0.935	0.985	0.135	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Small World: $\frac{CPl_{Normalised}}{CC_{Normalised}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.21

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Eglobal_{FA}$

$Eglobal_{FA}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-6.904E-5(0.000)	-0.072	-0.599	0.551			
Sex	5.612E-5(0.004)	0.002	0.016	0.987			
Diagnosis[a]	-0.011(0.005)	-0.411	-1.975	0.052			
AUDIT-C	0.000(0.001)	0.049	0.296	0.769			
Dx*AUDIT-C[b]	0.000(0.001)	0.097	0.433	0.666	0.991	0.156	0.002

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Eglobal: Global efficiency; $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.22

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on CPL_{FA}

CPL_{FA}							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	0.002(0.002)	0.106	0.880	0.382			
Sex	0.120(0.059)	0.274	2.045	0.045			
Diagnosis[a]	0.136(0.091)	0.314	1.498	0.139			
AUDIT-C	0.009(0.012)	0.132	0.797	0.428			
Dx*AUDIT-C[b]	0.002(0.018)	0.026	0.118	0.907	0.991	0.147	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. CPL: Characteristic path length; $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Table 4.23

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Strength_{FA}$

$Strength_{FA}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age	-0.003(0.008)	-0.038	-0.320	0.750			
Sex	-0.085(0.247)	-0.045	-0.343	0.733			
Diagnosis[a]	-0.7220.381)	-0.391	-1.894	0.063			
AUDIT-C	0.013(0.050)	0.043	0.263	0.794			
Dx*AUDIT-C[b]	-0.001(0.075)	-0.003	-0.012	0.991	0.991	0.169	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

4.5 Discussion

We sought to determine the association between the topological organisation of the brain and non-dependent alcohol use, and whether there was a differential relationship for people with a diagnosis of bipolar disorder. Alcohol was significantly related to subnetwork topology when weighted by the microstructural organisation of the fibre bundle, encompassing connections between fronto-limbic, basal ganglia and temporal nodes, and was not detected to have an effect globally on brain connectivity. Additionally, together alcohol and diagnosis were associated with a second subnetwork comprising cortico-limbic and basal ganglia connections, the strength of connectivity of this second subnetwork was negatively related to alcohol use in BD, this relationship was absent in controls.

4.5.1 Comparable Alcohol Use Between the Groups

There was no difference in alcohol use scores between the diagnostic groups, demonstrating that for our sample, people with a diagnosis of BD were not more likely to drink alcohol compared to healthy controls. Additionally, our data demonstrate that BD participants were not more likely than controls to consume alcohol in binge quantities, or to drink alcohol at potentially harmful amounts. Patients with BD report that they consume less alcohol when in a euthymic period, and more in manic or depressed phases (Meyer et al., 2012), our results can only be taken in the context of euthymic mood phase and cannot be extrapolated to depressive or (hypo)manic phases. Additionally, our data is limited as alcohol use is assessed in a self-report measure and may reflect an underestimation of our participant's alcohol use which could have implications for the results in both groups. However, our alcohol use scores are in line with those reported in a larger European study,

suggesting that these figures may be a reasonable and expected reflection of alcohol use within the groups (Lange et al., 2016).

4.5.2 Alcohol Use and Subnetwork Connectivity

We demonstrate that alcohol use is associated with a differently connected subnetwork of the brain involving regions responsible for reward, emotion regulation, and cognitive control processes which is not lateralised. The network elements (nodes and edges) are topologically arranged in a manner that changes with increasing alcohol use for all participants, resulting in greater connectivity strength of the subnetwork. Previous studies have demonstrated widespread lower cortical thickness and changes in subcortical volumes (Topiwala et al., 2017; Lange et al., 2016), we support these findings by providing evidence for subnetwork topology involving the areas implicated in these studies. Alcohol initially acts on reward circuitry stimulating an increase in the influence or salience of alcohol related clues and can therefore reinforce the likelihood of future alcohol consumption (Koob & Volkow, 2016). Nodes in the connected component associated with reward and salience processes are the caudate, putamen, and nucleus accumbens, and the insula (Koob & Volkow, 2016). These areas are proposed to be active during intoxication and interact with circuits involved in emotional regulation, such as amygdala and hippocampus to disrupt typical processing and regulation of emotions (Koob & Volkow, 2016). Additionally, circuits involved in goal directed behaviour through the regulation of the salience of environmental clues, and promotion of cognitive control are also impacted by alcohol use and are found in this subnetwork, these being prefrontal areas, hippocampus, and amygdala (Koob & Volkow, 2016). The transition from non-dependent alcohol use to habitual use involves repeated activation of circuitry heightening the salience and rewarding effects of alcohol, and disrupts processes associated with cognitive control (Naqvi & Bechara, 2010). As the AUDIT-C

assesses alcohol use over a twelve-month period the topological organisation of the subnetwork is not likely to be related to initial impacts of alcohol, and may reflect cumulative long-term use, these findings suggest that at a network level non-dependent alcohol use is associated with subnetwork alteration.

4.5.3 Topological Alteration in Bipolar Disorder with Alcohol Use

For BD participants there was an effect of alcohol on the topological configuration of an anatomical subnetwork involving the collective arrangement of nodes involved in cortico-limbic and reward networks, which was not evidenced in controls. The absence of a relationship between alcohol use and connectivity strength of the network in the control group, suggests a diagnosis-specific biological vulnerability for non-dependent alcohol use and may be consistent with the observed deleterious illness course associated with alcohol use in BD (Gordon-smith et al., 2020). Differential connectivity within reward and emotional circuitry related to BD diagnosis has been demonstrated (Nabulsi *et al.*, 2019), suggesting that pre-existing topological alterations may be driving the association with alcohol use within the BD group. Reductions in interhemispheric connectivity have been demonstrated in BD and are associated with alterations in cognition (Ajilore et al., 2015; Collin et al., 2016), our study demonstrates reductions in connectivity strength in specific interhemispheric connections with alcohol use in BD. This suggests that a pre-existing feature of the disorder may interact with alcohol use to place further stress on the network. Additionally, alterations in white matter microstructural organisation within the cingulum have been demonstrated in a large scale, multisite study in BD in comparison to controls (Favre et al., 2019). This white matter tract connects cortico-limbic nodes and is involved in emotional regulation and salience detection (Favre et al., 2019). These processes are compromised within the disorder (Tai et al., 2004), and again suggest that reductions in connectivity strength in the patterning

of this subnetwork may reflect initial biological vulnerability which is compounded by non-dependent alcohol use to contribute further to alter emotion and salience perception and increase the likelihood of relapse in the disorder. Network topology as well as the modular organisation of the network into intrinsic functional networks are found to support the optimisation of signal propagation through a network, thus influencing behavioural outcomes (Suárez et al., 2020). The interaction of network topology and dynamics support the flexibility of the network allowing for a rich behavioural repertoire capable of responding rapidly to environmental demands (Suárez et al., 2020). Providing initial descriptions of alterations to intrinsic functional networks in association with BD and non-dependent alcohol use may provide further insight into imbalance in between network connectivity, pointing to vulnerability to relapse in the disorder. A further exploration into the functional deviations arising from changes to topological organisation of the brain, may then be useful to identify possible compound impact to reward and emotion processing both independently in alcohol use and BD, and the interactive network effects.

There was an overlap of eight nodes (18%) between the two subnetworks: the hippocampus, caudate, pre and post central, entorhinal, lingual and supramarginal gyri and the pars opercularis, a number of these nodes are involved in reward and emotion processes. Of these common nodes, edge strength analysis reveals that the hippocampus, pars opercularis and precentral gyrus are associated with effects of greatest magnitude for BD participants. Reduced reward responsiveness of the hippocampus, precentral and inferior frontal gyri is demonstrated in BD in comparison to participants with unipolar depression and healthy controls (Redlich et al., 2015). Independently, alcohol use is associated with longitudinal cortical reductions in precentral and inferior/ medial frontal gyri (Meda et al., 2017) and of hippocampal volume, this decline is linked to poorer memory performance (Meda et al., 2019). Efficient reward processing is required to experience pleasure from

common experiences and stimuli, dysfunction within reward circuitry in BD contributes to mood lability and depressive episodes within the disorder (Redlich et al., 2015). Connectivity measures within this study are predicated upon recreated white matter tracts of the brain, while nodal information is important, our subnetwork analysis is based upon edge weight information. Therefore, the connections between these nodes are just as important as the nodes themselves. Dysconnectivity between frontal and temporal areas is thought to underlie aberrant functionality of emotion and reward processing within BD (Phillips & Swartz, 2014). This may be reflected by the reductions in connectivity strength within cortico-limbic circuitry demonstrated in this study, in particular between the hippocampus, precentral, and pars opercularis nodes which overlap between subnetworks. The connections between nodes and edges identifies some of the mesoscale subnetworks driving the emergence of complex behaviour, capable of responding effectively and efficiently to environmental stimuli (Bassett & Gazzaniga, 2011). The structure of these subnetworks will not define the function of the system, however, the adaption of the system to an environmental impact is likely to shape emergent complex behaviour going forward (Sporns, 2018b). This suggests that the environmental stimulus of non-dependent alcohol use may place additional stress on reward and emotion network interactions, thus contributing to a biological vulnerability to relapse within the disorder.

4.5.4. Topological Networks

The brain displays topological features giving rise to balance between integrated and segregated processing which allows the organism to switch between goal directed and automatic states (Shine & Poldrack, 2018). Global information integration in a network is essential to ensure that specialised messages from disparate sources are processed in parallel, this specialised information arises from segregated communities responsible for local

computation processes (Sporns, 2013). Disruptions to this topology may inhibit the effective transition between energy states and minimise the organisms' ability to efficiently interact with and respond to its' environment (Shine, 2019). We did not find any association between alcohol use and global connectivity measures suggesting that alcohol does not impact the brains' global topology, but alters the patterning of a network of subcomponents involved in reward and emotion-related processing. Previous connectivity research in AUD has demonstrated heterogenous findings, with some studies demonstrating reductions in global efficiency in association with increased duration of chronic alcohol use, and others reporting no difference between AUD and healthy controls (Zorlu et al., 2017; Sjoerds et al., 2015). An electroencephalographic study has demonstrated increased global efficiency and density of a resting state network, and reduced path length in association with acute alcohol use in social drinkers (Lithari et al., 2012). These alterations are suggested to facilitate communication thus allowing the effects of alcohol to spread more efficiently around the brain (Lithari et al., 2012). Conversely, this increase in efficiency is suggested to lead to reductions in global efficiency in chronic use due to damage associated with alcohol use (Lithari et al., 2012). This study can neither confirm nor deny these findings however, it is worth noting that an efficient network can facilitate disease progression, the same processes could also aid in damage to the architecture of the brain due to the toxic effects of alcohol use .

There is little doubt that chronic alcohol use negatively impacts the brain, however, what is less appreciated is the association between non-dependent alcohol use and physical, social, and legal harms. In 2018, 37% of adults who consumed alcohol in Ireland reported engaging in occasions of binge drinking, defined as consuming six or more standard drinks in one sitting (Department of Health, 2018). Within an Irish cohort, people who were not considered dependent on alcohol, and consumed alcohol at binge amounts occasionally and at least once a month reported an increased prevalence of harms to health, a negative impact on

finances, disruptions to study and impacts to social and personal relationships (O'Dwyer et al., 2019). Recent large-scale evidence suggests that there is no safe level of alcohol use (Topiwala et al., 2021), however, the impact of binge alcohol use in adult populations, on brain structure and function, as well as ensuing cognitive impacts remains unclear. This suggests that there are a group of people within our community who are underserved in terms of research on the potential damages to brain health in association with non-dependent alcohol use. Furthering research in this area would have a beneficial impact on the ability to apply neuroanatomical evidence to public health policy, strengthening the Irish government position commitment to reducing alcohol consumption and limiting the associated social and economic harms (Public Health Alcohol Act 2018).

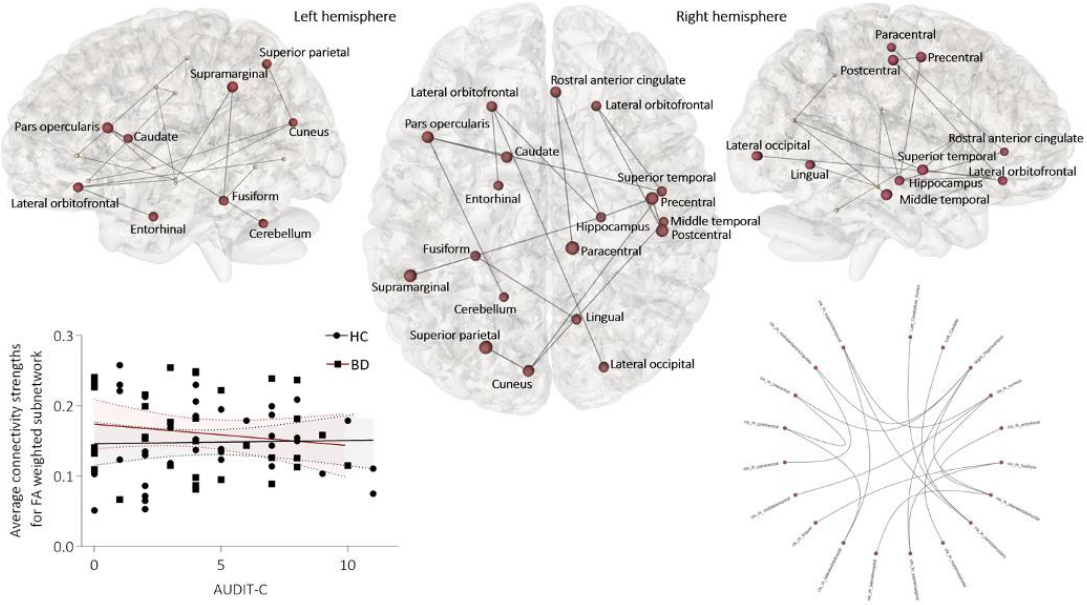
4.5.5 Conclusion

We demonstrate, in a first endeavour to understand the topological impacts of non-dependent alcohol use, subnetwork patterns of connectivity involving fronto-limbic, reward and temporal connections. Despite comparable amounts of alcohol use, we demonstrate a differential impact of alcohol use in BD, with decreased connectivity of a subnetwork containing cortico-limbic and reward connections apparent relative to control group. These subnetwork differences in BD may contribute to altered reward and emotion perception which heightens the likelihood of future alcohol use and increases vulnerability to relapse in the disorder.

Supplementary Methods and Results Study Two

Graphical Abstract

In bipolar participants relative to controls, connectivity negatively relates to alcohol use in a cortico-limbic, basal ganglia subnetwork.



Complex emergent behaviour is underpinned by the interaction of hierarchical networks in the brain (Sporns, 2018a). These networks appear at the mesoscale, between global and local measures, and are distinguishable by the emergence of community structures (Betzel & Bassett, 2017). By providing descriptions of the patterns of relationships which underlie sophisticated emergent behaviours, such as reward related activity and emotion regulation, it may be possible to better understand how and where alcohol use impacts the brain. We aim to examine the effect of moderate alcohol use within global network topology, to investigate if there is a subnetwork apparent, and if there is a differential effect in those with BD.

4.6 Supplementary Methods

4.6.1 Procedure for the Analysis of the Brain's Network

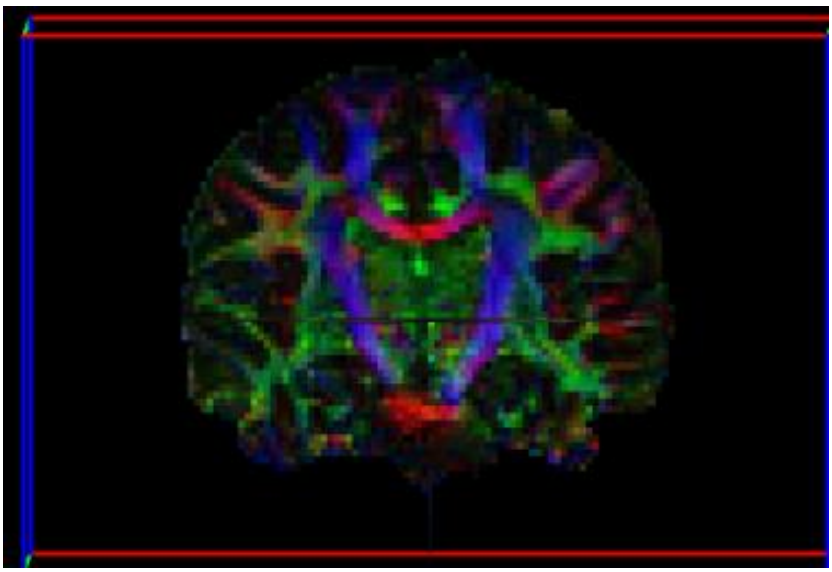
The following steps were used to create matrices to analyse properties of global integration and segregation.

Pre-processing diffusion images. Images were pre-processed to correct for eddy current distortions, motion artefacts, and susceptibility effects. Diffusion images are susceptible to artefacts due to involuntary and voluntary head movements in the scanner, cardiac pulsation, changes in the magnetic gradients, and the effects of bone and air on images (Jones, 2010).

Quality inspection of diffusion images. The quality of diffusion images for each participant was inspected to assess for artefacts which may affect data analysis. First eigenvector fractional anisotropy (FeFa) maps were inspected to ensure that axonal tracts corresponded to their correct anatomical orientation (*Supplementary Figure 4.4*).

Supplementary Figure 4.1

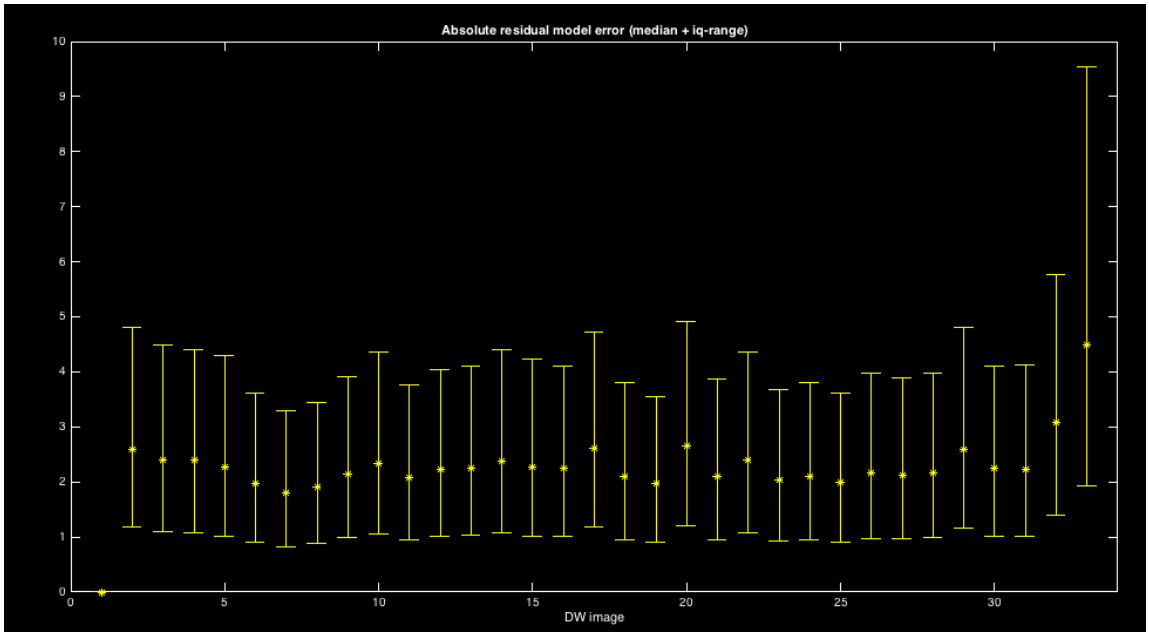
Screenshot of a FeFa Map with an Explanation of the Colours and Fibre Orientations of the Tracts



Each image was then inspected along each orthogonal view (axial, coronal, sagittal) by ‘looping’ the 33 images to visualize any distortions present in the images. These distortions may present as hyper-intensities, or zipper distortions. Residual graphs (*Supplementary Figure 4.5*), residual maps (*Supplementary Figure 4.6*), and outlier profiles (*Supplementary Figure 4.7*) were inspected for each image. The visualization and quantification of residuals creates an opportunity to identify where signal alterations may be present in the images which may be indicative of artefacts.

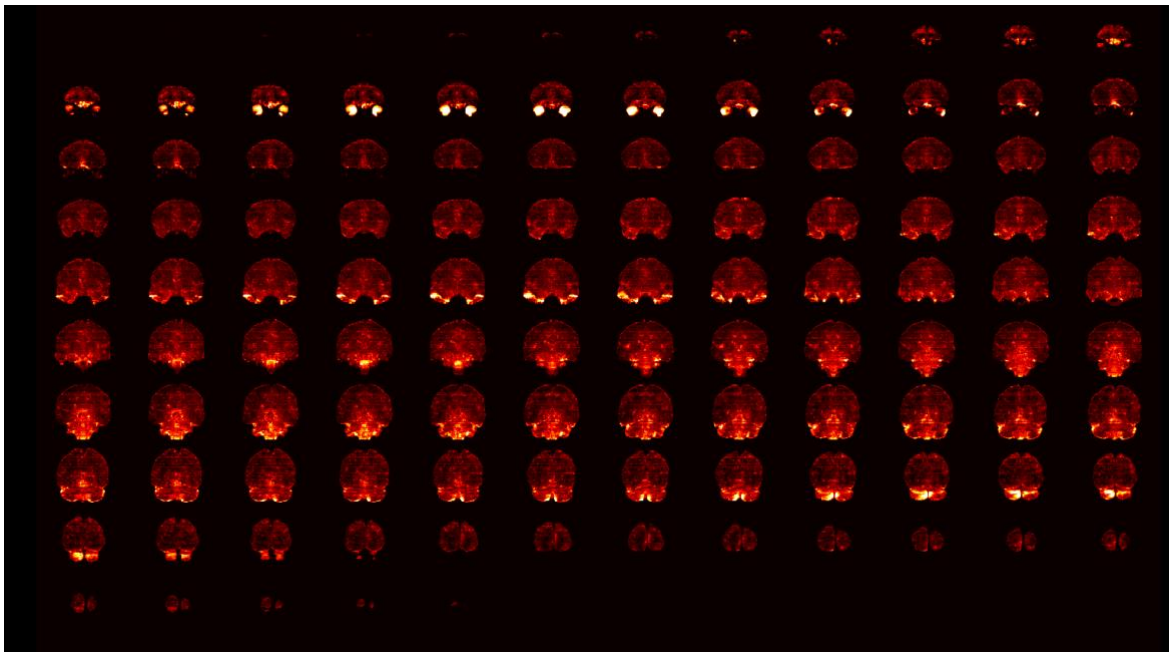
Supplementary Figure 4.2

Residual Graph Displaying Relatively Uniform Residual Distribution with One Peak at Slice 33



Supplementary Figure 4.3

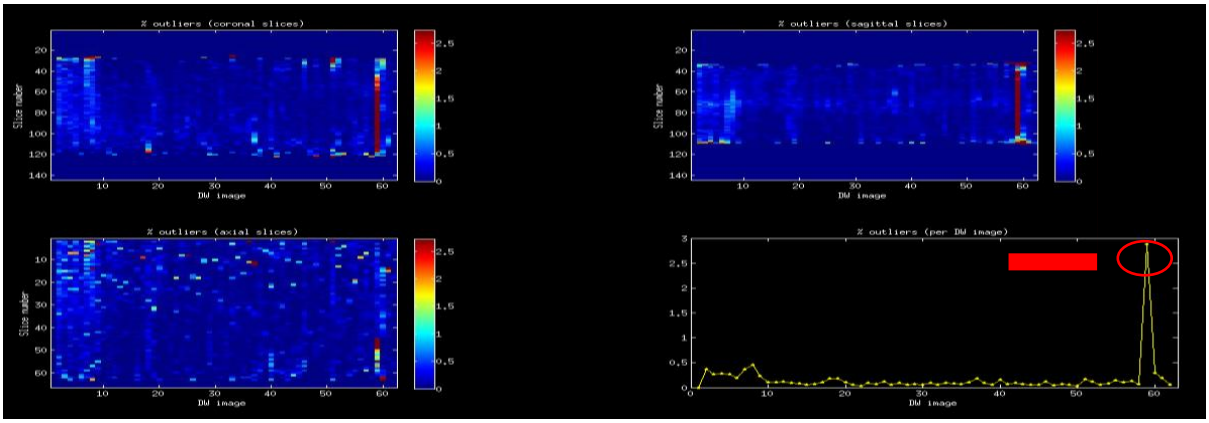
Residual Map at the Coronal View Displaying Hyper-intensities in the Temporal Lobes



The percentage of outliers present in each view was quantified for each image and presented in graph form. This displayed the approximate percentage of outliers present in each slice (*Supplementary Figure 4.4*).

Supplementary Figure 4.4

FA Outlier's Profiles Created for each Volume Slice in a Single Diffusion-Weighted Image in all Three Orthogonal Views

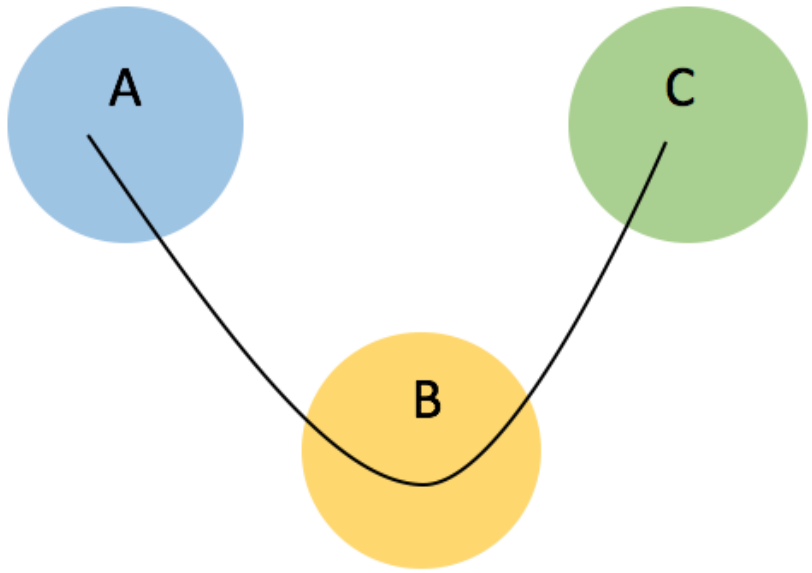


4.6.2 Generating Connectivity Matrices.

ExploreDTI (Leemans et al., 2009), creates structural connectivity matrices from node and edge information using the 'pass' protocol (*Supplementary Figure 4.8*). This ensures that edges will be reconstructed as they move through one node to another, this protocol allows for the integrative features of the network to be constructed and expressed as connectivity matrices (Hagmann et al., 2007). Utilising these matrices it is possible to investigate the brain as a graph and apply graph theory measures to describe integration and segregation of the network (*Supplementary Table 4.4).*

Supplementary Figure 4.5

A Depiction of the ‘Pass’ Protocol.



Note: This is a Connection that is Continuous from Node A, Through Node B, to Node C.

Supplementary Table 4.1

Description of Graph Theory Metrics Used to Investigate Brain Networks (Rubinov & Sporns, 2010)

Network parameter	**Description**
Global efficiency: E_{global}	The inverse of the average shortest pathlength in the network.
Characteristic path length: CPL	The average shortest path linking all nodes in the network.
Density	The fraction of present connections in relation to possible connections.
Strength	The average sum of weights of all nodes in the network.
Global clustering coefficient: CC	The average of all triangles present around all nodes of the network.
Normalised CC: γ	The ratio of the global CC to the averaged CC obtained from 100 matched random networks. A high γ indicates a higher level of network segregation.
Normalised CPL: λ	The ratio of the CPL to the averaged CPL obtained from 100 matched random networks. A network with low λ indicates a higher level of global network integration.
Small worldness: σ	Ratio between γ and λ, $\sigma > 1$ indicates the presence of a small world network.
Rich club coefficient	The fraction of edges that connect nodes of degree *k* or higher out of the maximum number of edges that may be shared by nodes.

4.7 Supplementary Results

While we controlled for age in our design we also demonstrate that there was no relationship between average FA in our sample and age at time of scan (r=0.034, p=0.774). Checks for distribution and outliers were undertaken, as well as multiple regressions using demeaned predictors to assess impact on the interaction model (Afshartous & Preston, 2011). In both instances the tests for multicollinearity suggest that there is no serious issue of correlation between the variables in the model. Testing to assess if the data met the assumption of collinearity indicated that multicollinearity was not a concern (Tolerance = 0.444, VIF = 2,254). The demeaned model is included in the supplemental regression reporting tables for comparison between both models (*Supplementary Tables 4.3-4.12).* It was decided at the time of manuscript preparation to report the main and interaction models without demeaned values as the variance in alcohol use scores and their association with cortical thickness was of primary interest. There was no difference found in significant results between the demeaned or original interactions.

The connectivity strengths for each suprathreshold connection in both subnetworks related to alcohol use are listed in *Supplementary Table 4.2*. The percentage change in BD figures demonstrate that having a diagnosis of BD and consuming alcohol results in decreases and increases in suprathreshold connections.

Supplementary Table 4.2

Suprathreshold Connections for Both Subnetworks

A: Suprathreshold Connections: AUDIT-C and Diagnosis	**Connectivity strength**		**Percentage**	**Magnitude of network**
	HC	**BD**	**change in BD**	**component effect (F-value)**
Left Cerebellum Cortex to Left Pars Opercularis	0.10±0.17	0.06±0.14	↓40	14.04
Left Caudate to Left Pars Opercularis	0.27±0.10	0.28±0.08	↑ 3.7	10.77
Left Cuneus to Left Superior Parietal	0.21±0.17	0.18±0.14	↓ 14	11.67
Left Cuneus to Right Superior Temporal	0.09±1.7	0.02±0.09	↓77	11.04
Left Cuneus to Right Middle Temporal	0.05±0.13	0.03±0.11	↓60	9.25
Left Entorhinal to Left Lateral Orbitofrontal	0.21±0.20	0.17±0.17	↓19	9.11
Left Fusiform to Left Supramarginal	0.10±0.10	0.13±0.20	↑30	10.00
Left Fusiform to Right Lingual	0.13±0.20	0.11±0.09	↓15	10.13
Left Lateral Orbitofrontal to Right Lateral Occipital	0.13±0.20	0.10±0.19	↓23	10.13
Left Pars Opercularis to Right Superior Temporal	0.17±0.23	0.10±0.19	↓41	9.48
Right Hippocampus to Right Precentral	0.24±0.17	0.18±0.17	↓25	12.50
Right Hippocampus to Right Rostral Anterior Cingulate	0.17±0.17	0.16±0.16	↓5.8	10.97

Right Hippocampus to Left Lateral Orbitofrontal	0.20±0.16	0.16±0.16	↓20	10.81
Right Hippocampus to Left Supramarginal	0.07±0.14	0.13±0.18	↑86	10.30
Right Lateral Orbitofrontal to Right Postcentral	0.19±0.20	0.16±0.19	↓16	8.90
Right Lateral Orbitofrontal to Right Superior Temporal	0.32±0.06	0.29±0.07	↓9.3	9.85
Right Paracentral to Right Rostral Anterior Cingulate	0.23±0.14	0.18±0.15	↓1.1	9.97
Right Postcentral to Right Precentral	0.21±0.14	0.23±0.11	↑9.5	9.07

B: Suprathreshold connections: AUDIT-C	**Magnitude of network component effect (F-value)**
Left Accumbens area to Left Temporal pole	11.01
Left Amygdala to Left Supramarginal	9.54
Left Amygdala to Left Temporal pole	10.44
Left Amygdala to Right Pars Orbitalis	8.64
Left Caudate to Left Frontal Pole	10.36
Left Caudate to Right Pars triangularis	10.44
Left Hippocampus to Right Lingual	9.70
Left Thalamus Proper to Left Precuneus	8.77

Left Thalamus Proper to Left Temporal pole	8.45
Left Thalamus Proper to Right Frontal Pole	11.40
Left Thalamus Proper to Right Precentral	9.02
Left Ventral DC to Right Superior Parietal	9.50
Left Ventral DC to Left Supramarginal	8.72
Left Banks STS to Right Postcentral	9.74
Left Entorhinal to Left Temporal pole	8.59
Left Entorhinal to Left Insula	11.78
Left Medial Orbitofrontal to Left Temporal pole	8.98
Left Medial Orbitofrontal to Right Postcentral	11.98
Left Parsopercularis to Left Precuneus	8.59
Left Pericalcarine to Right Rostral Middle Frontal	12.57
Left Supramarginal to Right Medial Orbitofrontal	15.05
Right Hippocampus to Right Precuneus	9.47
Right Caudate to Right Temporal Pole	9.36
Right Caudate to Left Frontal pole	19.19

Right Caudate to Left Insula	10.93
Right Putamen to Right Lingual	8.43
Right Putamen to Right Supramarginal	9.97
Right Putamen to Left Pericalcarine	8.74
Right Fusiform to Right Lingual	8.44
Right Fusiform to Right Precuneus	9.00
Right Isthmus Cingulate to Right Lingual	9.20
Right Isthmus Cingulate to Right Medial Orbitofrontal	8.45
Right Lingual to Right Precentral	8.64
Right Precentral to Right Supramarginal	8.87

Supplementary Table 4.3

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Eglobal_{Binary}$

$Eglobal_{Binary}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	5.858E-5(0.000)	0.030	0.257	0.798			
Sex	0.015(0.007)	0.283	2.193	0.032			
Diagnosis [a]	-0.015(0.006)	-0.275	-2.372	0.021			
AUDIT-C Demeaned	0.002(0.001)	0.218	1.366	0.177			
Dx*AUDIT-C Demeaned [b]	0.001(0.002)	0.087	0.529	0.599	0.985	0.208	0.003

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Binary: referring to presence or absence of connection; Eglobal: Global efficiency; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.4

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on CPL_{Binary}

CPL_{Binary}							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-6.871E-5 (0.000)	-0.024	-0.197	0.844			
Sex	0.003(0.011)	0.037	0.276	0.783			
Diagnosis[a]	0.032(0.010)	0.397	3.319	0.001			
AUDIT-C Demeaned	-0.001(0.002)	-0.050	-0.305	0.761			
Dx*AUDIT-C Demeaned[b]	0.002(0.003)	0.081	0.478	0.634	0.985	0.155	0.003

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Binary: referring to presence or absence of connection; CPL: Characteristic path length; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.5

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Density_{Binary}$

$Density_{Binary}$							
	Beta(SE)	β	*T*	$P_{Uncorrecte}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	1.590E-5(0.000)	0.008	0.065	0.948			
Sex	0.007(0.008)	0.119	0.902	0.370			
Diagnosis[a]	-0.021(0.007)	-0.366	-3.100	0.003			
AUDIT-C Demeaned	0.001(0.002)	0.113	0.698	0.488			
Dx*AUDIT-C Demeaned[b]	5.570E-5(0.002)	0.005	0.024	0.981	0.985	0.176	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Binary: referring to presence or absence of connection; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.6

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Clustering Coefficient

Clustering Coefficient							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-5.928E-5(0.000)	-0.048	-0.390	0.698			
Sex	-0.002(0.005)	-0.045	-0.333	0.740			
Diagnosis[a]	-0.013(0.004)	-0.338	-2.770	0.007			
AUDIT-C Demeaned	0.000(0.001)	-0.023	-0.136	0.892			
Dx*AUDIT-C Demeaned[b]	0.00(0.001)	0.031	0.179	0.858	0.985	0.121	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.7

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $CC_{Normalised}$ (γ)

$CC_{Normalised}$ (γ)							
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-0.001(0.001)	-0.237	-1.951	0.055			
Sex	0.015(0.023)	0.088	0.650	0.518			
Diagnosis[a]	0.054(0.020)	0.322	2.667	0.013			
AUDIT-C Demeaned	0.001(0.005)	0.033	0.199	0.843			
Dx*AUDIT-C Demeaned[b]	0.000(0.007)	0.003	0.019	0.985	0.985	0.138	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $CC_{Normalised}$: $\frac{CC_{Global}}{CC_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.8

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $CPL_{Normalised}$ (λ)

$CPL_{Normalised}$ (λ)							
	Beta(SE)	*β*	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-8.495E--5(0.000)	-0.129	-1.093	0.278			
Sex	-0.001(0.002)	-0.047	-0.357	0.722			
Diagnosis[a]	0.007(0.002)	0.411	3.493	<0.001			
AUDIT-C Demeaned	-5.630E-5(0.000)	-0.019	-0.117	0.907			
Dx*AUDIT-C Demeaned[b]	-3.752E-5(0.001)	-0.009	-0.051	0.959	0.985	0.181	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. $CPL_{Normalised}$: $\frac{CPL_{Binary}}{CPL_{Random}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.9

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on Small Worldness (σ)

Small Worldness (σ)							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-0.001(0.001)	-0.251	-2.065	0.043			
Sex	0.016(0.020)	0.106	0.784	0.436			
Diagnosis[a]	0.044(0.018)	0.304	2.507	0.015			
AUDIT-C Demeaned	0.001(0.004)	0.041	0.249	0.804			
Dx*AUDIT-C Demeaned[b]	0.000(0.006)	-0.014	-0.082	0.935	0.985	0.135	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Small World: $\frac{CPl_{Normalised}}{CC_{Normalised}}$; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.10

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Eglobal_{FA}$

$Eglobal_{FA}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-6.904E-5(0.000)	-0.072	-0.599	0.551			
Sex	5.612E-5(0.004)	0.002	0.016	0.987			
Diagnosis[a]	-0.009(0.003)	-0.334	-2.796	0.007			
AUDIT-C Demeaned	0.000(0.001)	0.049	0.296	0.769			
Dx*AUDIT-C Demeaned[b]	0.000(0.001)	0.074	0.433	0.666	0.991	0.156	0.002

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. Eglobal: Global efficiency; $_{FA}$: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.11

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on CPL_{FA}

CPL_{FA}							
	Beta(SE)	β	T	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	0.002(0.002)	0.106	0.880	0.382			
Sex	0.120(0.059)	0.274	2.045	0.045			
Diagnosis[a]	0.145(0.052)	0.335	2.783	0.007			
AUDIT-C Demeaned	0.009(0.012)	0.132	0.797	0.428			
Dx*AUDIT-C Demeaned[b]	0.002(0.018)	0.020	0.118	0.907	0.991	0.147	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. CPL: Characteristic path length; FA: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

Supplementary Table 4.12

Multiple Regression Examining the Interactive Effect of Alcohol and Diagnosis on $Strength_{FA}$

$Strength_{FA}$							
	Beta(SE)	β	*T*	$P_{Uncorrected}$	P_{FDR}	R^2	ΔR^2
Age Demeaned	-0.003(0.008)	-0.038	-0.320	0.750			
Sex	-0.085(0.247)	-0.045	-0.343	0.733			
Diagnosis[a]	-0.725(0.219)	-0.393	-3.315	0.001			
AUDIT-C Demeaned	0.013(0.050)	0.043	0.263	0.794			
Dx*AUDIT-C Demeaned[b]	-0.001(0.075)	-0.002	-0.012	0.991	0.991	0.169	0.000

Note: Variables used in these models have not been demeaned. [a]: difference in beta, *T* and multicollinearity values between demeaned and original figures found. [b]: difference in beta and multicollinearity values between demeaned and original figures found. FA: weighted by fractional anisotropy values; SE: Standard Error. ΔR^2 refers to the additional variance explained by the inclusion of the final variable.

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Chapter Five

Study Three: Alcohol use is Associated with Affective and Interoceptive Network Alterations in Bipolar Disorder

Author list: Fiona M. Martyn[1;2], Genevieve McPhilemy[1], Leila Nabulsi[1,3], Jacqueline Quirke[1], Brian Hallahan[1], Colm McDonald[1], & Dara M. Cannon[1].

Affiliations: [1]Centre for Neuroimaging & Cognitive Genomics (NICOG), Clinical Neuroimaging Lab, NCBES Galway Neuroscience Centre, College of Medicine, Nursing, and Health Sciences, National University of Ireland Galway, H91 TK33 Galway, Ireland. [2]School of Psychology, National University of Ireland, Galway. [3]Imaging Genetics Center, Mark and Mary Stevens Neuroimaging & Informatics Institute, University of Southern California, Marina del Rey, CA 90292, USA, Los Angeles, CA, United States.

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Authorship Confirmation Statement

FMM contributed to recruitment and data collection, performed MRI data quality checks, conducted all analyses and wrote the manuscript. DMC designed, obtained funding for and supervised data collection, analysis, and interpretation; BH and CMcD contributed to recruitment and intellectual content; LN and GMP recruited and collected data; JQ contributed to MRI data quality check.

Submitted to *Bipolar Disorders*

5.1 Abstract

Alcohol use in bipolar disorder (BD) is associated with mood lability and negative illness trajectory, while also impacting functional networks related to emotion, cognition, and introspection (Goldstein et al., 2006; Gordon-smith et al., 2020; Perry et al., 2018). The adverse impact of alcohol use in BD may be explained by its additive effects on these networks, thereby contributing to a poorer clinical outcome.

Forty BD-I (DSM-IV-TR) and 46 psychiatrically-healthy controls underwent T1 and resting state functional MRI scanning, and the AUDIT-C to assess alcohol use. Functional images were decomposed using spatial independent component analysis, into 14 resting state networks (RSNs), which were examined for effect of alcohol use and diagnosis-by-alcohol use accounting for age, sex and diagnosis.

Despite the groups' consuming similar amounts of alcohol (BD: mean score±SD 3.63±3.; HC 4.72±3, U=713, p=0.07) for BD participants greater alcohol use was associated with increased connectivity of the paracingulate gyrus within a default mode network (DMN) and reduced connectivity within an executive control network (ECN) relative to controls. Independently greater alcohol use was associated with increased connectivity within an ECN, and reduced connectivity within a DMN. A diagnosis of BD was associated with increased connectivity of a DMN, and reduced connectivity of an ECN.

Affective symptomatology in BD is suggested to arise from the aberrant functionality of networks subserving emotive, cognitive and introspective processes (Perry et al., 2018). Taken together, our results suggest that during euthymic periods, alcohol can contribute to the weakening of emotional regulation and response, potentially explaining the increased lability of mood and vulnerability to relapse within the disorder.

Keywords: alcohol use, functional connectivity, bipolar disorder, default mode network, executive control network

5.2 Introduction

Bipolar disorder is a chronic relapsing condition, characterised by fluctuations in mood state which affects approximately 1% of the world's population (Grande et al., 2016). Prevalence estimates of comorbid alcohol use disorders (AUD) in those with a diagnosis of bipolar disorder (BD) are between 30-35%, suggesting that there is a significant proportion of this group who may consume alcohol below clinical threshold for an AUD (di Florio et al., 2014). Moreover, those with a diagnosis of BD who do consume alcohol at non-dependent levels are more likely to experience increased lability of mood and thus relapse within the disorder than those who do not engage in alcohol use (Goldstein et al., 2006; Gordon-smith et al., 2020). The majority of research into the associations of resting state functional connectivity and alcohol use in BD has been undertaken in those with an AUD, however, this does not represent a complete spectrum of alcohol use patterns by people with a diagnosis of BD (di Florio et al., 2014). While alcohol at the levels used most broadly in society, may have some impact on functional connectivity (*Table 7.1*) (Hu et al., 2018), we hypothesise that its impact upon the already disrupted default, affective and executive control networks in individuals with BD (Sha et al., 2018; Syan et al., 2018) may be additive in its harmful impact and thereby contribute to poorer mood stability and clinical outcome in BD. Using a data driven model, this study will investigate the interactive effects of non-dependent levels of alcohol use on intrinsic resting state networks in BD relative to psychiatrically healthy controls.

In BD, reduced resting-state connectivity has been demonstrated between the amygdala, prefrontal cortex, the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) in BD, and is suggested to underlie mood dysregulation (Chase & Phillips, 2016) Alternatively, data driven approaches, such as independent component analysis (ICA) can be

used to decompose patterns of intrinsic functional connectivity into discernible networks thus avoiding a spatially restricted approach introduced by the selection of seed regions of interest (Calhoun, 2018). Historically preservation of intrinsic networks was suggested to be a feature of BD, with a review finding no difference in functional connectivity within networks decomposed through ICA (*Table 7.1*) (Syan et al., 2018). However, a recent large meta-analysis demonstrated increased and decreased functional connectivity within areas of the default mode network (DMN), sensorimotor, attention and visual networks within the disorder (Sha et al., 2018). Dysconnectivity within and between default mode, salience and cognitive networks modulating emotion processing is suggested to underlie symptoms of BD, with impairment in processing of emotional stimuli evident in the disorder (Perry et al., 2018). Moreover, altered functional connectivity between intrinsic networks has been demonstrated within the disorder, with increased connectivity between meso/paralimbic and fronto-temporal and right frontoparietal networks (Lois & Linke, 2014; Meda et al., 2012). This increase in connectivity between networks subserving affective and interoceptive processing is associated with increased negative symptoms (Meda et al., 2012). Moreover, longitudinal imaging studies suggest compensatory and decompensatory activation patterns within frontolimbic networks which relate to illness phase and mood state (Lim et al., 2013). Taken together, this suggests that vulnerability to relapse within the disorder may be mediated by connectivity alterations within and between resting state networks which disrupt affective, cognitive and introspective processes.

Research on resting state connectivity in association with non-dependent alcohol use is limited, however, reduced functional connectivity between the amygdala seed region and the right dorsal anterior cingulate cortex (ACC) has been demonstrated (*Table 7.1)* (Hu et al., 2018). Reduced functional connectivity in association with binge alcohol use is demonstrated between the amygdala and the orbitofrontal cortex (OFC), as well as between the inferior

frontal gyrus and hippocampus, areas involved in emotion and attention processes (Arienzo et al., 2020; Crane et al., 2018). Additionally, increased connectivity between striatal areas within the reward network and frontal areas involved in salience and attention networks are found in association with binge alcohol use (Arienzo et al., 2020). The increased connectivity demonstrated within the reward and salience networks are consistent with results found in AUD groups which may point to a greater motivation to consume alcohol.(Arienzo et al., 2020). Moreover, increased connectivity within nodes of the DMN was associated with increasing alcohol use in a sample of 25,378 UK residents, this study identified that the harms related to alcohol were more detrimental than those attributable to other modifiable factors (Topiwala et al., 2021). Recent studies within college-aged samples, have demonstrated increased connectivity in the left executive control network in association with binge alcohol use, and aberrant connectivity between the ventral attention network and right supramarginal gyrus (Herman et al., 2019; Sousa et al., 2019). Cycles of intoxication and withdrawal can lead to dysregulation of networks responsible for the effective processing of executive tasks, such as attention, inhibition, and goal orientated behaviour (Volkow et al., 2004). Prior work has also demonstrated alterations to neural structure: longitudinal samples have demonstrated that alcohol use is associated with reduced hippocampal volume and poorer cognitive outcomes in college-aged students and older adults (Meda et al., 2019; Topiwala et al., 2017). In conjunction with the findings in BD samples, these studies suggest that alcohol use may place BD participants at additionally vulnerability to aberrant connectivity within and between networks, in particular those associated with affective, cognitive and introspective processing.

The brain's functional network is underpinned by structural or anatomical architecture, the local and distributed topology of these networks constrains function while allowing for adaptability to environmental stimuli (Petersen & Sporns, 2015).The relational

architecture of these networks facilitates the emergence of cognitive processing, distributed and interactive mapping of anatomy onto computation supports a degenerative system and adaptability of function (Bassett & Sporns, 2017; M. Mesulam, 1990). The topology of functional networks changes throughout a person's life and is shaped by unique experience and learning processes, moreover, low levels of alcohol use are found to impact on neurovascular coupling leading to complex alterations and differential activation patterns across cortical regions (Bressler & Menon, 2010; Luchtmann et al., 2010). Correspondence between large scale intrinsic networks and behaviour is consistent across the literature (Laird et al., 2011), however, the requirement for degeneracy and adaptability of function means that coordinated patterns within local area networks can change dynamically (Bressler & Tognoli, 2006a). These dynamic patterns of local activity give rise to large scale intrinsic networks (Bressler & Menon, 2010). This suggests that function is dependent on the smooth interactions of local and large-scale networks, dysfunction at either scale can point to individual differences within the system (Bressler & Tognoli, 2006a). Spatial ICA is an ideal method to investigate the cooperation of local networks, through the assessment of coherence of activation within a large-scale intrinsic network, additionally it benefits from producing more biological and anatomically plausible results than temporal ICA providing increased sensitivity to identify between participant differences (Cole et al., 2010)

Alcohol use in BD is associated with mood lability and a poor clinical trajectory, and also with functional alterations to intrinsic resting state networks subserving affective, cognitive, and introspective processes (Goldstein et al., 2006; Gordon-smith et al., 2020; Herman et al., 2019; Sousa et al., 2019). We propose that non-dependent alcohol use may place those with a diagnosis of BD at vulnerability to relapse through compound alterations to connectivity within and between functional networks. We expect a differential impact of alcohol use on intrinsic connectivity for those with a diagnosis of BD relative to controls,

particularly in networks related to emotion, cognition, and introspection in addition to independent effects of use and of diagnosis on these core intrinsic networks' functional connectivity.

Table 5.1.

Studies Examining the Association of Alcohol or Bipolar Disorder with Functional Connectivity

Authors	***Sample size***	***Women: men***	***Age (years mean±SD)***	***MRI data analysis methods***	***Assessment of alcohol use***	***Findings***
Non-dependent alcohol use						
Hu *et al.*, 2018	N= 83	68:32	49±19	Rs fMRI, SBA: amygdala	NIDA Quick Screen	↓ connectivity right dACC and amygdala, stronger relationship in men.
Potential hazardous alcohol use						
Vergara *et al.*, 2017	N=188	38:62	33±9	Rs fMRI, ICA	AUDIT	↓ FC between SN, SM, precuneus and visual networks. ↑ FC between reward and visual networks.
Binge alcohol use						
Arienzo *et al.*, 2020	N= 35	54:46	25±4	Rs fMRI, SBA	AUDIT	↑ connectivity between striatum, ACC, and OFC. ↓ connectivity between IFC and hippocampus.
Sousa *et al.*, 2019	N=34	52:48	20±2	Rs fMRI, ICA	AUDIT	↑ FC in left ECN.

Herman, *et al.*, 2018	N= 30	70:30	23±5	Rs fMRI, ICA	Alcohol Use Questionnaire	↓ FC in VAT.
Crane *et al.*, 2018	N= 46	21:89	26±4	Rs fMRI, SBA: amygdala	Timeline Follow Back	↓ FC between right amygdala and OFC.
Bipolar disorder						
Syan *et al.*, 2018	N=23 studies	-	-	Review	Not asked	↔ within network alterations. Aberrant FC between amygdala, cingulate, medial, and ventrolateral prefrontal cortices.
Sha *et al.*, 2018	N=242 studies	41:59	29±11	Review	Not asked	↓ FC in areas of SMN, DMN, DAN, VAT ↑ FC in areas of DMN, DAN, VAT, visual networks.
Vargas *et al.*, 2013	N= 8 studies	-	-	Review	Not asked	Largest differences in FC within limbic-striatal network
Du *et al.*, 2015	N=93	54:46	34±11	Rs fMRI, ICA	Not asked	Insula cortex as discriminative region for BD.
Khadka *et al.*, 2013	N=374	53:47	38±13	Rs fMRI ICA	Not asked	↑ FC in meso/paralimbic and ↓posterior DMN in patients. ↓ FC in fronto-occipital, frontal/thalamic/basal ganglia, and

						↑ SMN networks in patients and first-degree relatives.
Meda *et al.*, 2012	N=374	53:47	38±13	Rs fMRI ICA	Not asked	↑ FC between MPN and fronto-temporal networks. ↓ FC between fronto-occipital and anterior DMN/prefrontal.
Öngür *et al.*, 2010	N=46	43:57	39±10	Rs fMRI, ICA: DMN	Not asked	↓ FC within mPFC, abnormal recruitment of parietal cortex.
Lois *et al.*, 2014	N=65	57:43	41±9	Rs fMRI, ICA	Not asked	↑ FC between MPN and right FPN.

Note: ACC: anterior cingulate cortex; BD: Bipolar disorder; B-YAACQ: Brief Young Adult Alcohol Consequences Questionnaire; dACC: dorsal anterior cingulate cortex; DAN: dorsal attention network; DMN: default mode network; DMQ-R SF: Drinking motive questionnaire revised short form; DRN: drinking only; ECN: executive control network; FC: functional connectivity; FPN: frontoparietal network; ICA: Independent component analysis; IFC: inferior frontal cortex; mPFC: medial prefrontal cortex; MPN: Mesoparalimbic network; NIDA: National Institute on Drug Abuse; OFC: orbitofrontal cortex; PACS: Penn Alcohol Craving Scale; Rs fMRI: resting state functional magnetic resonance imaging; SBA: seed based analysis; SMN: sensorimotor network; SMAST: Short Michigan Alcohol Craving Scale; VAT: ventral attention network

5.3 Methods

5.3.1 Participants

Participants to the study met with a registered psychiatrist for a Structured Clinical Interview (SCID), patient or control edition, a diagnosis of bipolar disorder type-I or type-II was confirmed using the DSM-V-TR criteria (American Psychiatric Association, 2000). All participants were between the ages of 18-65, control participants (HC) were admitted to the study if they had no diagnosis of an Axis-I disorder, no first degree relative with a confirmed mental health diagnosis, and no current or historical use of medication for anxiety or depression. Exclusionary criteria for all participants were: a history of an alcohol use disorder, a loss of consciousness lasting more than five minutes, pregnancy or breastfeeding, a current gastrointestinal disorder, any heart problems, or uncontrolled blood pressure. All participants provided written consent; ethical approval was granted by The Galway University Hospital Clinical Research Ethics Committee.

5.3.2 Assessment of Alcohol Use

Alcohol use data was collected via the AUDIT-Consumption (AUDIT-C), which consists of the first three questions of the larger 10 question AUDIT, it is validated for use in a variety of settings (Bush et al., 1998). The scale measures frequency and amount of alcohol use over the previous 12 months, as well as the frequency of binge alcohol use occasions ($\geq$6 standard drinks in one episode). Each item is scored 0 to 4, with a maximum score of 12, a score of $>$5 on the AUDIT-C indicates a potential for harmful use (Long & Mongan, 2013). A history of AUD excluded a participant from the study, this assessment was made by a registered psychiatrist during a Structured Clinical Interview.

5.3.3 Assessment of Clinical Signs and Symptoms

The Hamilton Depression Rating Scale (HDRS) objectively and reliably quantifies depressive episodes (Hamilton, 1960; Trajkovi et al., 2011)[;]. The scale is clinician rated and comprises 24 questions, scoring is based on the first 17 items, with a range of 0-53, a score of ≤8 indicates the absence of symptoms of a depressive episode (Hamilton, 1960). The Young Mania Rating Scale (YMRS) is an 11-item scale which is valid and reliable for the identification of (hypo)manic episodes (Young & Meyer, 1978). Scoring is based upon objective clinical ratings during a clinical interview, a score of <7 indicates absence of (hypo)manic symptoms in the BD participants (Chengappa et al., 2003; Young & Meyer, 1978)[·] Absence of symptoms related to depressive mood or (hypo)manic symptoms in the participants defines a participant as euthymic.

5.3.4 MRI Acquisition

MRI data for all participants was obtained using a high-resolution 3T Achieva scanner (Philips Medical Systems, Netherlands) at the Wellcome Trust Health Research Board Centre for Advanced Medical Imaging (CAMI), at St James's Hospital, Dublin, Ireland. A high resolution 3-dimensional structural T1-weighted *Magnetization Prepared Rapid Acquisition Gradient Echo* (MPRAGE) sequence was acquired using an 8-channel head coil (echo time (TE) 3.9 ms; repetition time (TR) 8.5 ms; flip angle 8°; 1 mm^3 isotropic voxel size, 180 slices). Resting state data was acquired using a single shot gradient echo planar imaging (EPI) sequence, involving whole-brain acquisition of 180 volumes (repetition/echo times = 2000/28 ms, flip angle 90^o, field of view 240 X 240 X 133 mm, 3 X 3 mm voxel dimensions, 80 X 80 matrix size, and 38 axial slices of 3.2 mm each). Resting state scans were acquired while participants were supine with eyes open and instructed to remain fixed on a crosshair for the 6-minute scan duration.

5.3.5 Subject Level Image Preprocessing

Motion correction of MR images was undertaken using a six-parameter rigid body transform and interpolated using a fourth-degree b-spline. Corrected images were inspected to assess translation and rotation parameters, all motion correction was within the bound of one voxel, with no spikes in motion greater than half a voxel. Within subject co-registration of structural to functional images was undertaken using a rigid-body model and image reslicing. Images were again inspected to ensure anatomically accurate alignment for each participant. Structural images were segmented to create maps of grey, white, and CSF tissue types, with a bias corrected image created. This image was then spatially normalised using affine transformations. Functional and structural images were normalised to the Montreal Neurological Institute (MNI) template. Images were spatially smoothed with a Gaussian kernel of 6 mm at full width half maximum. Pre-processing of fMRI data was undertaken using Statistical Parametric (SPM12) software (Wellcome Centre for Human Neuroimaging) (Friston et al., 1994).

5.3.6 Group Level Spatial Independent Component Analysis

A model order of 30 was chosen to ensure spatial stability of derived components with RSNs replicated in literature (Abou-Elseoud et al., 2010). Group component extraction was undertaken using the Infomax algorithm, which was repeated twenty times in ICASSO to maximize the stability of the decomposed components (Jafri et al., 2008). Group level IC's were back reconstructed using GIG-ICA, a method which obtains subject specific ICs with stronger independence and spatial correspondence across subjects, as well as increased accuracy (Du & Fan, 2013). All steps related to spatial ICA were undertaken using the GIFT Toolbox (Calhoun et al., 2001).

5.3.7 Selection of Resting State Networks

Two researchers (FMM and JQ) independently assessed each IC to classify the components as likely signal related to resting state networks (RSNs) or noise related to motion, physiological processes, or low signal drift. A component was classified as an RSN if it displayed a low number of large clusters with peaks of activation located in grey matter, had a regularity of time course oscillations with power spectra in the low frequency range, was spatially correlated with grey matter templates in SPM8 and had a higher ratio of grey matter in comparison to white matter and cerebrospinal fluid. (Griffanti et al., 2017). Potential networks were cross referenced with networks which have been replicated in the literature.(Laird et al., 2011) Neuroanatomical locations of significant signal changes were obtained by overlaying masks of the RSN images onto the MNI template. The Harvard-Oxford cortical and subcortical atlas was used to label regions based on cytoarchitectonic probabilities, which was accessed through the SPM Anatomy toolbox (Eickhoff et al., 2005).

5.3.8 Functional Connectivity and Statistical Analysis

We analysed three distinct but complementary aspects of functional connectivity to describe within and between-network connectivity in relation to alcohol and diagnosis, these being: spatial map intensity, BOLD power spectra, and functional network connectivity (Allen et al., 2011). The spatial maps of each RSN were analysed to identify differences in the participation of a voxel or cluster of voxels in a network which relates to the level of connectivity and degree of coactivation within a network (Allen et al., 2011). The power spectra of RSN timecourses relating to the level of coherent activity at a specific frequency within a network were analysed by estimating each BOLD spectrum based on detrended subject-specific timecourses, using a multi-taper approach: a bandwidth of three and number

of tapers set to five using the Mancovan toolbox (Allen et al., 2011). Functional network connectivity relates to the connectivity between networks of interest, component timecourses were band-pass filtered with a Butterworth filter with cutoff frequencies of 0.008-1.5 Hz to reduce the likelihood of signal unrelated to RSNs (Allen et al., 2011). Relationships between spatial map intensities, BOLD spectral power, functional network connectivity, and independent variables: diagnosis, AUDIT-C score, and an interaction between diagnosis and AUDIT-C score, controlling for age, sex and motion parameters were assessed using the Mancovan toolbox in GIFT. An alpha level of 0.05 was used for all analyses, with results corrected for multiple comparison using the false discovery rate (FDR). Group differences in demographic and clinical data were assessed using Chi-squared for categorical data (sex, hazardous use, and binge alcohol use), and a *t*-test for normally distributed data (age). Non-normally distributed data was assessed using the Mann-Whitney U (AUDIT-C, Hollingshead Scale, HDRS, TMRS). Statistical significance was assessed using a two-tailed α level of 0.05 (SPSS s v.24 IBM Corp., New York, USA).

5.4 Results

5.4.1 Sample Demographic and Clinical Characteristics

A total of 86 individuals participated in this study, 40 participants with a diagnosis of BD (33 BD-I, 7 BD-II) and 46 healthy controls. As the groups were matched for age and sex, no significant differences were found (*Table 5.2*). A significant difference was found between the groups in scores on the HDRS (U=339.5, p<0.000), and the YMRS (U=678.5, p=0.016). The majority of the BD participants were euthymic at the time of scanning (n=28, 70%). A statistically significant difference was found in socioeconomic status, BD participants were more likely to report a lower status in comparison to control participants (U=583.5, p=0.004).

Table 5.2

Clinical and Demographic Characteristics of the Sample

	Healthy controls n=46	*Bipolar participants n=40*	*Statistical Comparison Test statistic, p*
Sex (f:m, n)	30:16	22:18	χ^2=0.934, 0.381
Age at MRI (years)	41.00±14	43.08±13	T=-0.721, 0.473
SES status (mean±SD)	42.22±16	31.52±16	U=583.5, 0.004*
HDRS (mean±SD)	1.04±1.7	6.78±6.6	U=339.5, 0.000*
YMRS (mean±SD)	0.72±1.5	1.80±2.6	U=678.5, 0.016*
Lithium use (no, %)	0, 0	26, 65	-
Antipsychotic use (no, %)	0, 0	13, 33	-

Note: Socioeconomic status was assessed using the Hollingshead Scale. AUDIT- C: Alcohol Use Disorders Identification Test-Consumption; f: female; HDRS: Hamilton Depression

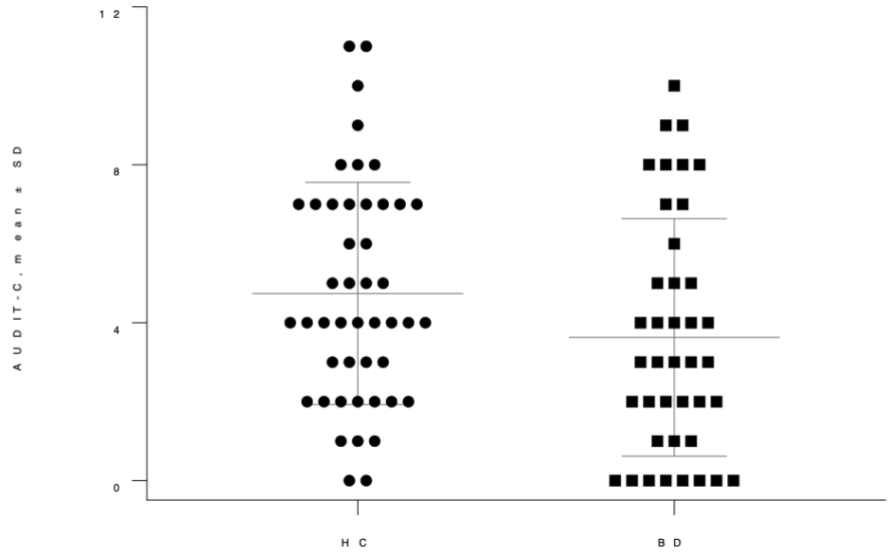
Rating Scale; m: male; YMRS: Young Mania Rating Scale. Data are presented as mean$\pm$sd.

*significant at α=0.05.

5.4.2 Comparable Alcohol Use Scores Between the Groups

There was no difference between the diagnostic groups in alcohol use scores (*Figure 5.1*). Forty six percent of controls and 35% of BD participants reported scores that indicated a possibility for harmful alcohol use, however this difference was not statistically significant between the groups (*Figure 5.1).* Additionally, the two groups did not differ significantly in the reporting of their frequency of engaging in binge drinking episodes, that is consuming six or more alcoholic drinks in one sitting (Bush et al., 1998). The reported AUDIT-C scores represent a wide range of non-normally distributed alcohol use scores within our participants, ranging from no alcohol use (a score of 0) to a potential for consuming alcohol daily or almost daily (maximum score 11). There was no association between alcohol use and SES scores for HC (r= -0.164, p=0.277) or BD (r=0.087, p=0.593).

Figure 5.1. No Difference in Alcohol Use scores Between the Groups



	Healthy controls *n=46*	*Bipolar participants* *n=40*	*Statistical comparison* *Test statistic, p*
AUDIT-C (mean±SD)	4.74±2.81	3.63±3	U=713.5, *p*= 0.072
Positive for hazardous drinking (n,%)[a]	21 (46)	14 (35)	$\chi 2$=1.006, p= 0.316
Frequency of binge (n)[b]	Never: 15 Less than Monthly: 16 Monthly: 5 Weekly: 10	Never: 19 Less than Monthly: 11 Monthly: 5 Weekly: 5	$\chi 2$=2.658, *p*= 0.447

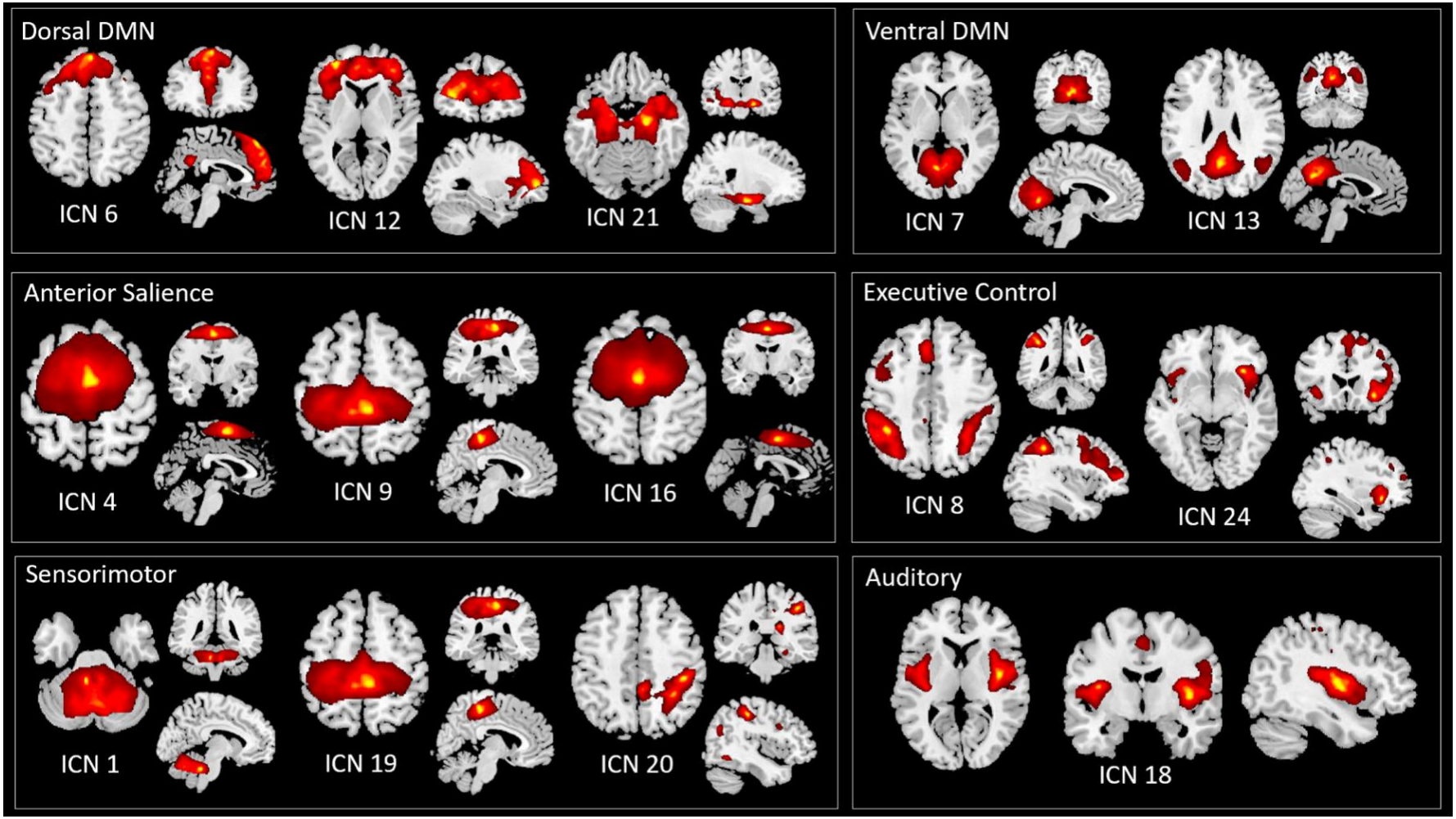
Note:[a]:Hazardous use is defined as scoring >5 in the AUDIT-C. [b]:A binge is defined as drinking more than 6 standard drinks in one setting.

5.4.3 Resting State Networks Within the Data

Following decomposition into 30 networks, one ICN (30) was not retained due to low stability analysis as determined by the cluster quality index I_q>0.938. After manual classification 16 ICNs were deemed noise as activation occurred within areas of white matter and cerebral spinal fluid space, time courses were irregular, displayed power spectra within frequency bands aligned with cardiac pulsation or respiratory noise, or had low correlation with grey matter templates in SPM8. Of the remaining 14 components (*Figure 5.2*) correspondence with templates from Shirer et al., (2012) was established to describe the RSNs anatomically as: dorsal DMN (RSNs 6, 12, 21), ventral DMN (RSNs 7, 13), anterior salience (RSNs 4, 9, 16), executive control (RSNs 8, 24), sensorimotor (RSNs 1, 19, 20), and auditory networks (RSN 18).

Figure 5.2.

Resting State Networks Obtained Through Independent Component Analysis



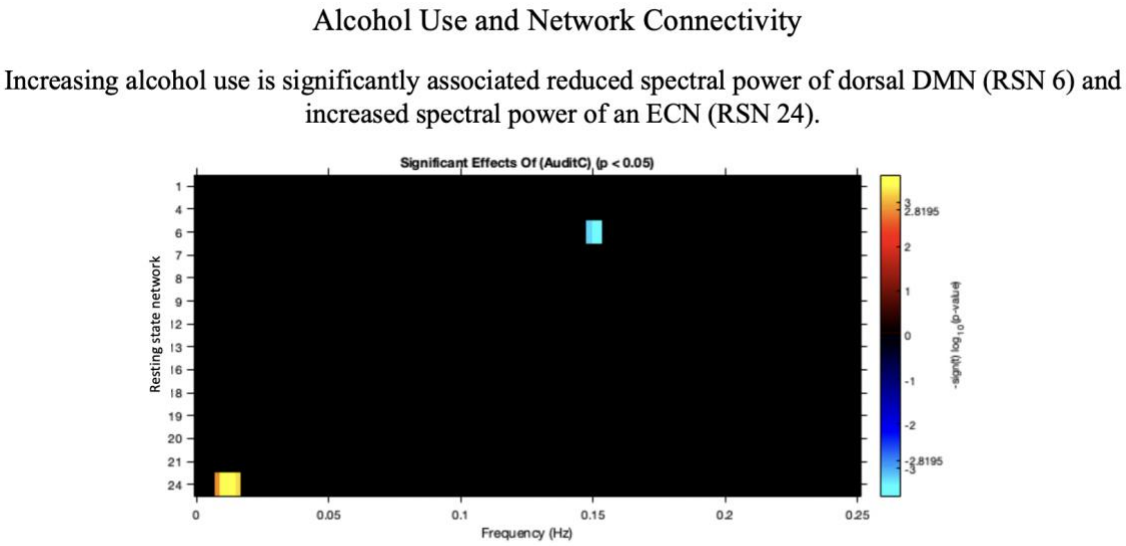
Note: Resting state networks derived from spatial ICA are overlaid on a canonical brain thresholded at false discovery rate $p<0.05$. RSN number is displayed beneath, network classification is derived from Shirer *et al.* 32012. DMN: default mode network; RSN: resting state network. Activation thresholded at $T>20$.

5.4.4 Altered Resting State Connectivity in Relation to Alcohol Use and a Diagnosis of Bipolar Disorder

Coactivation within networks or between network connectivity was not associated with alcohol use in this sample. However, spectral analysis demonstrated that greater alcohol use was associated with reduced BOLD spectral power at 0.15 Hz in a dorsal DMN network, and greater BOLD spectral power within the low frequency range (>0.05 Hz) in an executive control network (*Figure 5.3*).

Figure 5.3.

Alcohol is Significantly Associated with Altered Connectivity Within Default Mode and Executive Control Networks

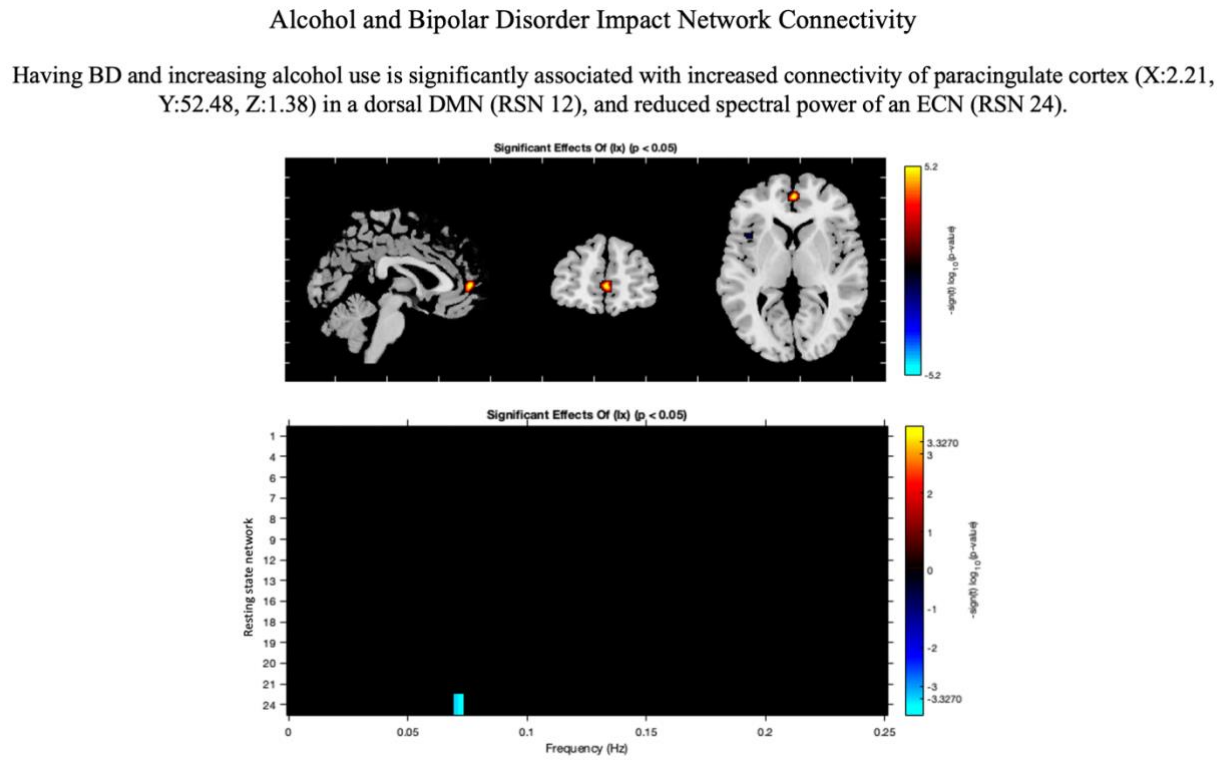


Note: In all figures red/ orange indicates a positive relationship and blue a negative, p-values corrected using the false discovery rate. DMN: Default Mode Network; RSN: Resting State Network.

Among participants with a diagnosis of BD, greater alcohol use was associated with a statistically significant increase in connectivity of the paracingulate cortex within a dorsal DMN (RSN 12, *Figure 5.4; Supplementary Figure 5.4*). A significant decrease in BOLD spectral power, between 0.05- 0.1 Hz within an executive control network (RSN 24) was also detected (*Figure 5.4*). In contrast, connectivity between the examined networks did not relate to alcohol consumption in HC or BD groups.

Figure 5.4.

A Diagnosis of Bipolar Disorder with Increasing Alcohol Use is Significantly Associated with Altered Connectivity within Default Mode and Executive Control Networks

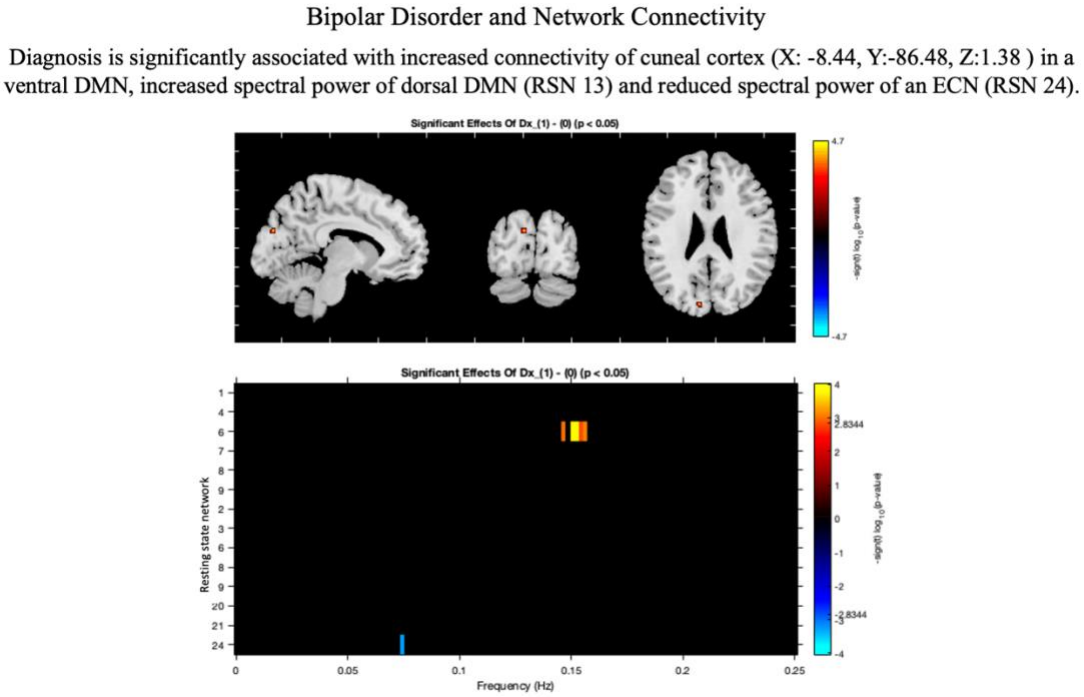


Note: In all figures red/ orange indicates a positive relationship and blue a negative, p-values corrected using the false discovery rate. DMN: Default Mode Network; RSN: Resting State Network.

A diagnosis of BD was associated with a statistically significant increased connectivity of the cuneal cortex within the ventral default mode network (RSN 13) (*Figure 5.5).* Additionally, a significant decrease in BOLD spectral power within an ECN (RSN 24) between 0.05- 0.1 Hz was detected for participants with a diagnosis of BD, with greater BOLD spectral power within a dorsal default mode network (RSN 6) at 0.15Hz (*Figure 5.5).* A diagnosis of BD did not relate to connectivity between the examined networks.

Figure 5.5.

A Diagnosis of Bipolar Disorder is Significantly Associated with Altered Connectivity within Default Mode and Executive Control Networks



Note: In all figures red/ orange indicates a positive relationship and blue a negative, p-values corrected using the false discovery rate. DMN: Default Mode Network; RSN: Resting State Network.

5.5 Discussion

Using the data driven multivariate approach of independent component analysis, we sought to determine the impact of alcohol on intrinsic resting state networks and if there was a differential impact for people with a diagnosis of BD. We demonstrate that increasing alcohol consumption was associated with alterations to the level of coherent activity at specific frequencies within default mode and executive control networks. We find that having a diagnosis of BD, but moreover having a diagnosis of BD and consuming alcohol is significantly associated with increased connectivity within default mode networks and reduced spectral power at 0.05-0.1 Hz in an executive control network. These results suggest that introspective and cognitive networks are impacted by alcohol, over and above BD alone, altered functionality related to emotion processing subserved by these networks may contribute to vulnerability to relapse within the disorder.

5.5.1 Measuring the Spatial Organization of the Brain

Seminal work by Biswal et al., (1995) demonstrated that the brain displays distinct regional synchronous fluctuations in intrinsic activity, led to an explosion in the investigation of functional connectivity (Poldrack, 2018). Functional studies typically characterise connectivity by the degree of coactivation within a network or by the connectivity between networks, however, by investigating the spatial organisation of brain oscillatory activity it may be possible to describe with more depth the functional organisation of the brain (Baria et al., 2011). Unimodal brain areas which encode basic features of sensation transmit information to transmodal limbic or paralimbic areas (Mesulam, 1998). This increase in complexity corresponds to changes in power distribution where unimodal areas are dominated by low frequency power and transmodal by high frequency power distributions

(Baria et al., 2011). We demonstrate that for participants with BD who engage in alcohol use there is a reduction in the coherence of oscillatory activity between 0.05-0.1 Hz within an ECN. This may reflect reduced engagement of the network at a particular frequency, or a compensatory mechanism in association with alcohol use within the disorder. Previous work has demonstrated that in comparison to control participants, patients with a diagnosed mood disorder displayed less low-frequency spectral power in a dorsal DMN which was associated with life-long suicidal ideation (Malhi et al., 2020). These results suggest a that a depressed state may be associated with a pattern that differs from what we would have detected in our primarily euthymic sample. Taken together they demonstrate the relevance of investigating BOLD power spectra in higher-order mood disorders to better understand the spatial organisation of brain oscillatory activity.

5.5.2 Alcohol and Functional Connectivity

We demonstrate that in the presence of increasing alcohol use there was a reduction in the coherence of oscillatory activity at 0.15 Hz in a DMN and a greater coherence of oscillatory activity at a low frequency (>0.5 Hz) in an ECN for all participants in this study. A recent large-scale prospective cohort study demonstrated increased functional connectivity within the default mode network in a dose-dependent manner (Topiwala et al., 2021). The average alcohol intake for this sample was 13.5 standard alcoholic drinks per week, despite this being in keeping with current UK government guidelines the results suggest that even low levels of alcohol use are harmful to the brain, potentially requiring a rethink of guidelines. Our demonstrated results in the DMN support these findings, while expanding on them to include alterations in a cognitive network. Previous work by Vergara *et al.* (2020) (mean AUDIT score= 15.3 ±5.3) and Sousa *et al.* (2019) (11.2 ±3.25) report functional alterations within and between networks supporting cognitive and emotional processes,

which supports our results in an ECN. The studies by Vergara *et al.* (2020) and Sousa *et al.* (2019) report AUDIT scores which are significantly higher than the average score for our sample (5.45 ±4.9), together with the recent work by Topiwala *et al* (2021) this suggests that functional alterations of intrinsic networks are apparent at a range of alcohol consumptions which are common in our communities. The impact of alcohol on the brain depends on numerous molecular mechanisms, which in turn can be reflected in differences of activation as measured by the BOLD response (Luchtmann et al., 2010). Previous work has demonstrated that a low amount of alcohol does not distort simple repetitive movements, but suggests that more complex movements or behaviours may be impacted due to the decrease of neuronal activation, as well as the potential for the loss of neurovascular coupling due to alcohol induced vasodilation (Luchtmann et al., 2010). Moreover, it suggests that circuitry involved in affective or cognitive functions may be more susceptible to alcohol (Luchtmann et al., 2010), which supports the findings of this study that non-dependent alcohol use impacts activation coherence in cognitive and introspective networks.

5.5.3 Functional Connectivity in Bipolar Disorder with Alcohol Use

We found increased connectivity of the paracingulate cortex in a dorsal default mode network and reduced coherence of oscillatory activity at 0.5-1.0 Hz within an ECN for participants with a diagnosis of BD who consumed alcohol, in comparison to healthy controls. The DMN comprises a large set of co-activated brain areas that form a system for self-monitoring, autobiographical thought, and perceiving the perspectives of others (Buckner et al., 2008). The ECN is proposed to be responsible for high-level cognitive processes, for instance orientating attention and identifying emotional stimuli (Bressler & Menon, 2010). The DMN and ECN engage in discrete cognitive processes and guide responses to emotionally salient stimuli (Bassett & Gazzaniga, 2011). A recent meta-analysis

demonstrated reduced within-network connectivity of a network corresponding to our ECN, for patients in an acute mood state in contrast to those with the disorder in a remitted state (Wang et al., 2020). These reductions in connectivity in the ECN are suggested to reflect instabilities of mood which are prominent within the disorder, with a normalisation of connectivity in the remitted state reflecting improved function and affective processing (Wang et al., 2020). We demonstrated reduced connectivity within the ECN for those with a diagnosis of BD who are predominately euthymic while controlling for alcohol use within the sample. The lack of control for confounding factors may have influenced the findings of Wang *et al.* (2020) and suggests that alcohol may be an important factor which has not been adequately controlled for in previous research. Moreover, we demonstrate compound alterations within the ECN for those with a diagnosis of BD in the presence of increasing alcohol use. We suggest that the compound effect of alcohol consumption and a diagnosis of BD impacts on the coherence of oscillatory activity within this RSN, potentially weakening emotional control during euthymic periods. Changes in functional connectivity, as demonstrated by reduced BOLD spectral power of the ECN, combined with increased connectivity within the DMN may point to vulnerability in identifying and responding to emotionally salient internal and external stimuli in BD in association with alcohol use, thus impacting on vulnerability to increased mood lability in the disorder.

5.5.4 Functional Connectivity in Bipolar Disorder

We demonstrate increased functional connectivity of the cuneal cortex and greater coherence of oscillatory activity at 0.15 Hz within a default mode network and reduced coherence of oscillatory activity at 0.05-1.0 Hz within an executive control network in association with a diagnosis of BD. Reduced functional connectivity within the DMN during acute mood episodes in contrast to hyperconnectivity within DMN in remitted mood states

has been previously reported in BD relative to healthy controls (Wang et al., 2020). Alterations of connectivity within the DMN are suggested to reflect heightening planning in relation to internal and external environments for those with a diagnosis of BD (Wang et al., 2020). The majority of our sample were euthymic at the time of scanning, and our results support this distinction in DMN connectivity. Moreover, alterations of decreased connectivity within a ventral default mode network have been demonstrated within BD and for their first-degree relatives (Khadka et al., 2013), our results support changes within the connectivity of a ventral DMN within BD and provide evidence for functional alterations which may impact interoceptive processes within the disorder. The DMN can be subdivided into regions, while this may be a function of dimension reduction, frequency of oscillatory activity within the DMN is demonstrated to change from low frequency in posterior to high frequency in anterior portions (Baria et al., 2011). This points to a functional heterogeneity within the network and challenges the idea that the entire network is suppressed in association with task demands (Buckner et al., 2008). Our results identify ventral and dorsal components of the DMN and demonstrate alterations to within network connectivity for these subcomponents of the network. The successful suppression of the DMN is critical for cognitive operations, such as attention and emotional response, processes which are known to be compromised within BD (Buckner et al., 2008). Our demonstrated increase in DMN activation may reflect difficulties in suppression and orientation towards cognitively controlled emotional regulation, leading to the exacerbation of mood dysregulation seen within the disorder.

We have demonstrated alterations in power spectra within dorsal DMN and an ECN for people with a diagnosis of BD. Low-frequency oscillations have been used to identify large scale networks and to study their organisational properties (Baria et al., 2011). The largest power spectra for the DMN is located within the low-frequency, with the posterior portion of the DMN dominated by the low-frequency, and frontal portions showing

oscillations at higher frequencies, with a suggestion that areas of the brain responsible for more complex processes are dominated by higher frequencies (Baria et al., 2011). Within our ECN is contained the insula, a structure which supports switching between the DMN to the ECN, so that attention is orientated towards cognitively controlled states (Bressler & Menon, 2010). Participants with a diagnosis of BD demonstrate deficiencies in switching from internally focused processes to task related processing in the presence of cognitive-affective stimuli (Ellard et al., 2019). This occurs through increased activation within the DMN and reduced activation of the insula, suggesting that BD participants are less able to disengage from self-monitoring processes in the presence of emotional distractors, and move to a cognitively controlled state (Ellard et al., 2019). Our findings of increased DMN activation and reduced power of activation within an ECN support these findings, suggesting that the coherent activity of the networks is disrupted within the disorder, and may point to network vulnerability to relapse, possibly exasperated, we suggest, by the consumption of alcohol. The reductions in coherent activation within intrinsic networks suggest that brain states reflect dynamics, whereby interactions between frequency oscillators are critical aspects of these dynamics, which are embedded within the anatomical structure of the brain. This then reflects the need to approach fMRI or connectivity from a dynamic perspective, rather than single point estimate of function. Observing energy requirements to transitions between brain states, and the alterations that may be present in BD in association with alcohol use will provide a richer explanation of the dynamic system of the brain within the disorder. This work contributes to previous knowledge by pointing to specific network patterning that is altered within the disorder, which may influence the regulation of interoceptive processes and the engagement of networks supporting cognitively controlled emotional processing.

5.5.5 The Brain as a Network

A challenge to studying BD is the intricate and diffuse symptoms reported among patient groups, the use of data-driven approaches can be beneficial as they analyse whole brain function and can identify weak contributions from numerous regions which may point to specific disorder related pathology (Calhoun, 2018). Applying this multivariate method may provide more sensitive evidence of the impact of alcohol use in BD and its contribution to mood lability and relapse within the disorder and contributes to the identification of mechanisms related to the disorder. Interactions within functional networks give rise to cognitions and behaviours required to navigate ever changing social environments, within-network connectivity can draw on dynamic interactions of varying subsets of the network which coordinate their activity, depending on the function required (Bressler & Menon, 2010). Specific operations require the co-activation of a specific pattern of integrated local area networks to give rise to the required process, alteration within this patterning of network integration could lead to dysfunction (Bressler & Menon, 2010; Bressler & Tognoli, 2006b). This may be reflected in the alterations of within-network connectivity in association with a diagnosis of BD and alcohol use and may reflect aberrant self-referential processes and difficulty in engaging local areas networks for emotional regulation. Each local area subnetwork makes its own specific contribution towards the cognitive function of the network; therefore, a local cortical area is multifunctional: it is the patterning and coherency of activation that calls on specific function within a repertoire of functionality (Bressler & Menon, 2010). While a local area network may express activities related to more than one function, the integrated functional expression of contributions of all local area networks are combined within the large-scale network. Therefore, subtle dysfunction within local areas can lead to alterations in the smooth functioning of the large-scale network (Mesulam, 1990). Our results demonstrating alterations to coherent within-network connectivity in DMN and ECN

may point to dysfunction of the smooth operation of the functioning of the networks, leading to mood dysregulation and impacting on the likelihood of relapse within the disorder in the presence of alcohol use.

5.5.5 Limitations to the Study

The interpretation of resting state networks is challenging as there is currently no global agreement on naming conventions, or dimension reduction for networks. We chose to decompose our data to 30 components with the aim of aligning the spatial correspondence between our networks and those reliably replicated in the literature (Abou-Elseoud et al., 2010). This model order was a trade-off between large scale and finer grained network components, as we were interested in networks which often appear at the finer level, for example salience network, and also large-scale networks, that of the DMN. A limitation to our study is that alcohol use has been recorded as a self-report measure. However, the AUDIT-C has been validated for use across a variety of setting and is a well-used tool in research studies (Bush et al., 1998).

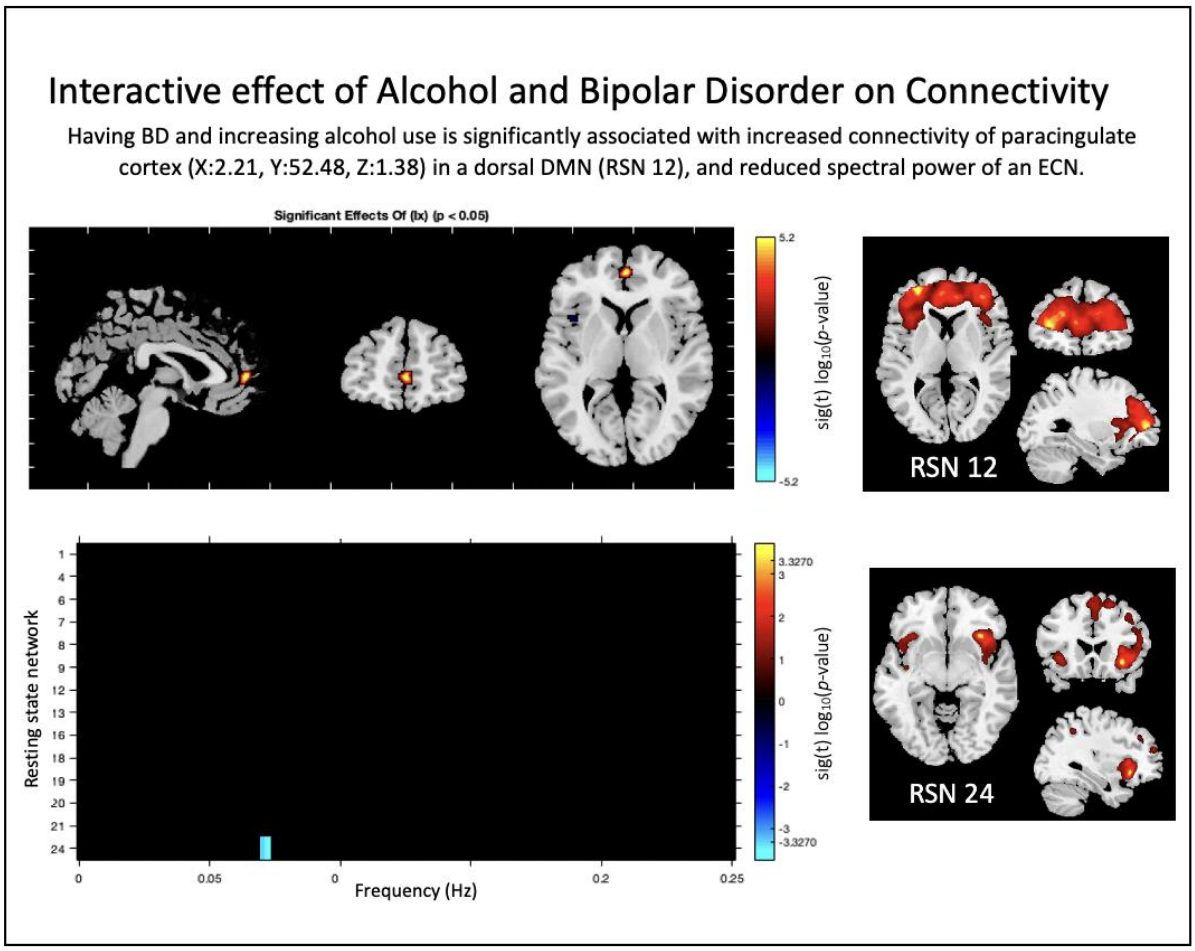
5.5.6 Conclusion

In a first endeavour to understand the functional impact of non-dependent alcohol use in bipolar disorder, we demonstrate alterations to within-network connectivity of interoceptive and cognitive networks, which are involved in the regulation of emotion. Despite the groups consuming comparable amount of alcohol, we demonstrate alterations to default mode and executive control networks for people with a diagnosis of BD, relative to healthy controls. This alteration may impact on previously identified deficiencies in switching from internally to externally focused tasks during emotion processing, which results in a lack of ability to disengage in self-monitoring processes in BD (Ellard et al.,

2019). These alterations to within network connectivity suggest that even during euthymic periods, alcohol can contribute to the weakening of emotional regulation and response, potentially explaining the increased lability of mood and vulnerability to relapse within the disorder.

Supplementary Methods and Results Manuscript Three

Graphical Abstract



Independently alcohol use and bipolar disorder (BD) are associated with functional network alterations (Hu et al., 2018; Syan et al., 2018). This suggests that people with a diagnosis of BD may demonstrate a differential connectivity profile in association with non-dependent alcohol use in comparison to healthy controls. This study aims to identify alterations within and between intrinsic resting state networks in association with non-dependent alcohol use, particularly for people with a diagnosis of BD.

5.6 Supplementary Methods

The following outlines the parameters that were used to decompose the resting state fMRI data into intrinsic networks, this was undertaken using GIFT software (Calhoun et al., 2004).

Supplementary Figure 5.1

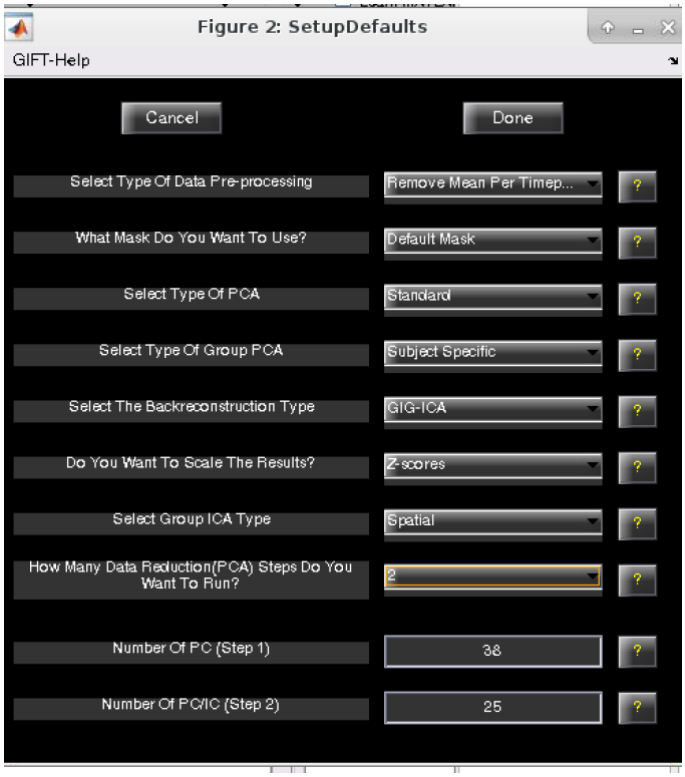
Parameters of GIFT Software

To constrain the number of networks the data will be decomposed into do not choose to estimate the number of ICs. A general rule of thumb is that 20-30 IC's will give the most reliable results. You will need to choose the type of algorithm that you want to use, in this case I have selected Infomax (Figure X.1). You will need to choose the type of stability analysis that you wish to use, in this case I have chosen ICASSO, this will determine the reliability of the ICA algorithm selected by running it a number of times. I have chosen GIG-

ICA as my back reconstruction method and have left the other selections at the defaults (Figure 8.2).

Supplementary Figure 5.2

Parameter Selection for Spatial ICA in GIFT software

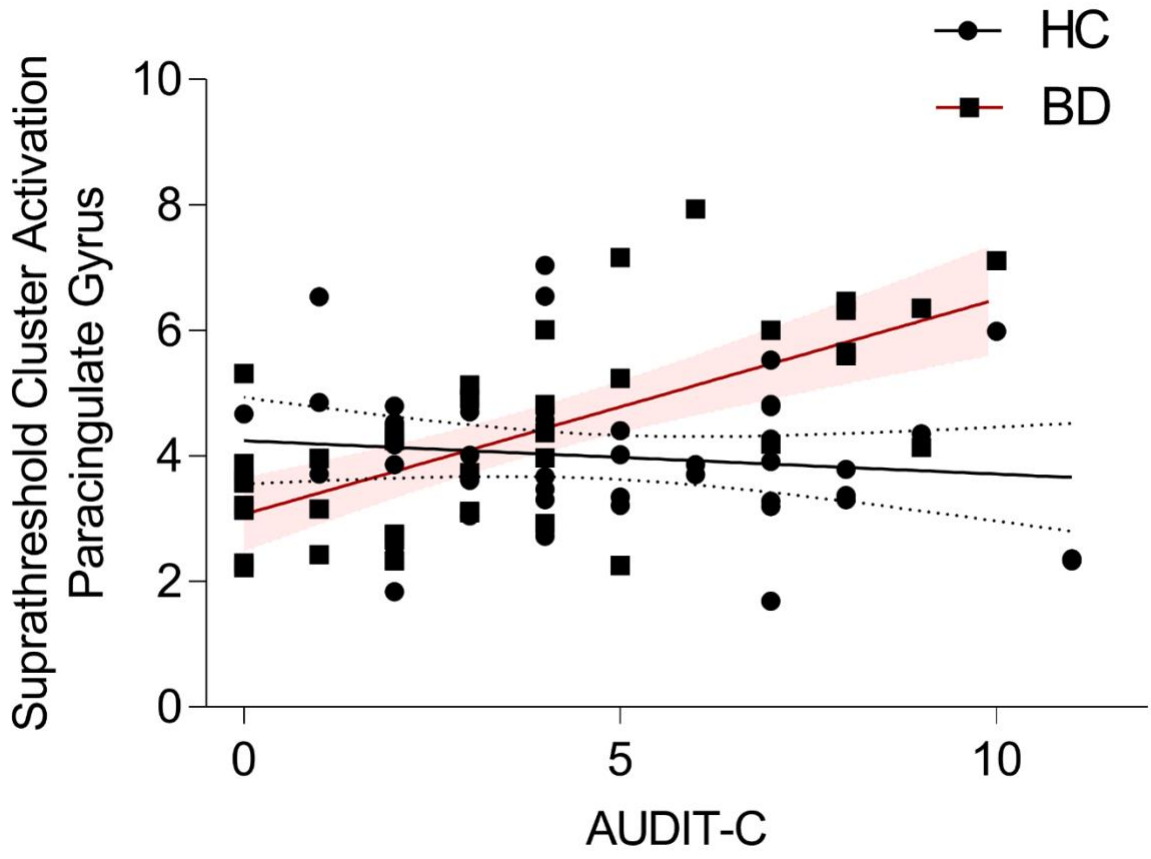


5.7 Supplementary Results

After demonstrating that there was a statistically significant interaction in the paracingulate cortex *post hoc* testing was undertaken to be sure that this result was being driven by the BD participants, *Supplementary Figure 8.3* demonstrates that this is the case.

Supplementary Figure 5.4

Functional Activation of the Paracingulate Gyrus is Increased for Those with a Diagnosis of Bipolar Disorder who Consume Alcohol



5.10 References

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Chapter Six

Thesis Discussion

6.1 Introduction

While alcohol has a widespread impact on the brain, it is likely that there are specific circuitry involved in emotion and reward processes which may be more vulnerable to its toxic effects (Monte & Kril, 2015). Moreover, structural and functional MRI research in BD identifies structural alterations in emotion and reward networks as well as aberrant reward and emotion processes in this disorder (Guadalupe et al., 2017; Hibar et al., 2018; Strakowski et al., 2012). This suggests that together having a diagnosis of BD and consuming alcohol may place the individual at increased vulnerability to structural and functional alteration in specific circuitry, which may lead to relapse in the disorder. This thesis investigated the neuroanatomical impacts of alcohol use particularly for people with a diagnosis of bipolar disorder, and capitalised on technological advances in network analysis within its application to *in vivo* neuroimaging. To achieve this T1-weighted, diffusion-weighted and resting state MRI was used to identify associations between structural and functional alterations and alcohol use scores. This portion of the thesis will build on the points emanating from the three manuscripts (*Table 6.1*), these being: Alterations to specific reward network structures and alcohol use in BD, preferential subnetwork connectivity patterns with alcohol use in BD, and intrinsic network connectivity alterations in association with alcohol use in BD. Strengths and limitations of the methods employed by the thesis will be identified, with suggestions for future directions for this work in the area of neuroscience and the potential impact on health policy in Ireland.

6.1.1 Study One

This study used T1-weighted structural MRI to investigate alterations to the cortical thickness of specific reward related structures, namely: bilateral dorsolateral prefrontal, anterior cingulate, orbitofrontal and insula cortices in association with alcohol use. Using this method it is possible to identify alterations at relatively high resolution which may be attributable to alcohol use. It is demonstrated that alcohol use was associated with reduced cortical thickness of the left anterior cingulate, orbitofrontal, and insula cortices for all participants. Additionally, it is shown that having a diagnosis of BD and consuming alcohol was associated with reduced cortical thickness of the left anterior cingulate cortex and dorsolateral prefrontal cortex. Moreover, between group testing demonstrated that reduced cortical thickness of the left dorsolateral prefrontal cortex was only found in BD participants. These results identify structural alterations in association with alcohol use to prefrontal areas of cortex that exert top-down control over reward and emotion processing (Buschman et al., 2014). In particular, the dlPFC is involved in moderating reward expectancies and exercising cognitive control over emotional responses (Beylergil et al., 2017), these processes are demonstrated to be compromised in BD (Price & Drevets, 2012). The contribution of alcohol use to pre-existing structural alterations attributable to a diagnosis of bipolar disorder may influence the functional outcomes of the network and reflect aberrant reward and emotion processing in BD, leading to mood lability in the disorder.

6.1.2 Study Two

This study investigated structural neuroarchitecture in association with alcohol use using T-1 and diffusion-weighted MRI and graph theory analysis. This paper advances from a regional grey matter structural investigation to one predicated upon the grey and white matter topology of the brain, bringing the added advantage of conceptualising the brain as a complex

network. It is demonstrated that alcohol use is associated with increased connectivity for all participants in a subnetwork comprising fronto-limbic, basal ganglia and temporal nodes. Moreover, the study finds an additional subnetwork which is differently connected for participants with BD in comparison to healthy controls. This subnetwork is associated with reduced connectivity strengths of nodes involved in cortico-limbic and basal ganglia circuitry. There is no association with alcohol use and global measures of integration and segregation. These results suggest that cumulative use of alcohol is associated with differential impacts to subnetworks for all alcohol consumers and for those with a diagnosis of BD independently. Previous work in BD has demonstrated a dysconnected subnetwork involving connections between limbic and basal ganglia nodes, regions which support reward and emotion processes (Nabulsi et al., 2019). These results suggest that pre-existing subnetwork alterations may be placed at additional vulnerability to the impact of alcohol use, which is reflected in connectivity alterations between cortico-limbic and basal ganglia structures. This altered pattern of connectivity may impact on reward expectancies and emotion processing, thus influencing emotional lability within the disorder.

6.1.3 Study Three

Moving from static structural investigations, this paper investigated functional connectivity of the brain, resting state fMRI data was decomposed into intrinsic functional networks using independent component analysis to assess the association between network connectivity and alcohol use. This data-driven multivariate analysis has the power to detect weak signal contributions from multiple sources which impact on functional connectivity within and between intrinsic networks (Calhoun, 2018). It is found that increasing alcohol use is associated with altered patterns of connectivity within DMN and ECN networks. Additionally the results demonstrate that having a diagnosis of BD, and moreover having a

diagnosis of BD and consuming alcohol is associated with aberrant functional connectivity within default mode and executive control networks. The DMN and ECN guide the processes of identifying and responding to internally and externally emotionally relevant stimuli (Bassett & Gazzaniga, 2011). Aberrant introspective processes have been linked to an inability to disengage from emotional stimuli in BD and respond in a cognitively controlled manner to obtain relevant rewards (Perry et al., 2018). This work suggests that alcohol contributes to dysfunction in networks subserving the identification of emotionally relevant stimuli and appropriate cognitively controlled responses, thus contributing to mood lability and the potential for relapse in the disorder.

6.2 The Main Findings of the Thesis

Taken together these papers suggest that both groups: control and BD participants demonstrated alcohol related differences in brain structure and function, and that even though there was no statistically significant difference in alcohol consumption scores between the groups, BD specific pattern of changes were also observed, with functional relevance to reward and cognitive processes. Previous work has suggested that alcohol use has a distributed impact on the brain (Lange et al., 2016) for all consumers and also those with BD, while this may be the case, this thesis identifies that discreet cortical and subcortical regions, both individually and as part of distributed networks are impacted by alcohol, which has the potential to influence reward and emotion processing.

Table 6.1

Neuroanatomical findings related to alcohol use in bipolar disorder

Study 1	***Anatomical location***	***Association with alcohol***	***Functional networks***
	Left anterior cingulate, orbitofrontal, and insula cortices	↓ Thickness all participants	Reward, limbic, cognitive control
	Left dorsolateral prefrontal cortex	↓ Thickness BD only	Reward, cognitive control
Study 2			
	Left: accumbens, temporal pole, amygdala, supramarginal, caudate, frontal pole, hippocampus, thalamus, precuneus, ventral DC, banks STS, entorhinal, medial orbitofrontal, pars opercularis, pericalcarine, frontal pole, insula. Right: pars orbitalis, pars triangularis, lingual, precentral, superior parietal, postcentral, rostral middle frontal, medial orbitofrontal, hippocampus, precuneus, caudate, temporal pole,	↑ Connectivity strength all participants	Reward, salience, limbic, cognitive control

putamen, supramarginal, fusiform, isthmus cingulate, precentral.		
Left: cerebellum, pars opercularis, caudate, cuneus, superior parietal, entorhinal, lateral orbitofrontal, fusiform, supramarginal. Right: hippocampus, superior temporal, middle temporal, lingual, lateral occipital, superior temporal, precentral, rostral anterior cingulate, lateral orbitofrontal, postcentral, paracentral.	↓ Connectivity strength BD only	Reward, limbic cognitive control
Global integration and segregation	↔ Effect	
Study 3		
Paracingulate cortex.	↑ Connectivity strength BD only	Default mode network
Executive control network	↓ Connectivity strength BD only	Cognitive control
Dorsal default mode network	↓ Connectivity strength all participants	Default mode network

Executive control network	↑ Connectivity strength all participants	Cognitive control

6.3 Alcohol Use is Associated with Alterations of Reward and Affective Circuitry

Reward circuitry mediates aspects of behavioural reinforcement and value representation associated with internal and external stimuli, this process relies on interactions between various brain circuits interconnected by neurotransmitter systems such as dopamine, glutamate, serotonin and GABA (Volkow & Morales, 2015). Reward processing is not a unitary construct and encapsulates numerous aspects such as pleasure, motivation, salience, anticipation and satiety which shape behaviour and decisions (Whitton et al., 2016). Aspects of reward are often conceptualised in an affective sense: 'liking' or hedonic responses during the consumption of a pleasurable stimulus, and in incentivizing contexts: 'wanting' or motivational processes which occur during the anticipation of reward (Pujara & Koenigs, 2014). The affective contributions of reward can occur explicitly, that is in the conscious awareness of the person, however, they have also been linked to implicit behaviours, where a person will react to a reward without awareness of the stimulus or their hedonic reaction (Berridge & Robinson, 2003). Learning is a key cognitive process which guides reward behaviour, knowledge is required for reward prediction, for guidance by cues and for goal directed behaviour, thereby increasing the likelihood of reward predicated behaviour occurring in the future (Berridge & Robinson, 2003).

The affective experience of reward is underpinned by circuitry which includes the prefrontal, orbitofrontal, anterior cingulate and insular cortices, as well as subcortically, the nucleus accumbens, ventral pallidum and amygdala (Berridge 2015). These areas act together to pay attention to the stimulus, appraise the stimulus, act on previous memories of the stimulus, and make a decision to behave (Berridge 2015). We demonstrate that for all participants consuming alcohol is associated with reduced cortical thickness in these medial and frontal cortical structures (Study One), as well as altered structural connectivity in a subnetwork containing these nodes (Study Two), moreover, we find that functional

alterations are present in networks containing these regions (Study Three). This suggests that consuming alcohol is associated with changes to how stimuli are perceived and acted upon. Our finding of increased connectivity within the ECN, which involves the anterior cingulate and insula are particularly interesting in this context, as they provide evidence that consuming alcohol is associated with alterations to networks subserving attentional and affective processes.

Experience or awareness of emotion is suggested to be guided by representations of interoceptive sensations associated with limbic regions of the brain for instance the temporal lobe, anterior cingulate, orbitofrontal and insula cortices, and exteroceptive sensations which are associated with sensory areas of the brain (Lindquist & Barrett, 2012). The process of making meaning of these sensations using associations from past experience within changing contexts is associated with the DMN function, while the regulation and orchestration of the construction is underpinned by frontoparietal networks (Lindquist & Barrett, 2012). This suggests that emotions signal our deepest motivations and desires, prompt adaptive responses while experiencing an emotion, and elicit an adaption of behaviour while viewing or receiving the emotional response of another (Fox, 2018). Therefore, while emotions are private internal experiences, they also have a social component that is external to the private milieu of the person. The findings of altered structural connectivity of a subnetwork containing regions supporting reward and emotion processing (Study Two), as well as the complex interplay of increased connectivity patterns within an ECN and reduced connectivity patterns within a DMN (Study Three) in association with alcohol provides an interesting support for these theories. In the context of these findings, consuming alcohol is associated with a weakening of the connectivity of the DMN, which may lead to difficulties updating perceptions in changing contexts, as well as regulating and acting upon the construct in hand. Studies of acute alcohol use demonstrate impairments in divided and focused attention, as

well as visuomotor control and reaction time (Zoethout et al., 2011) suggesting that alcohol intoxication impairs the performance of cognitive and introspective networks' ability to attend to stimuli and update behaviours accordingly. This has support in neuroimaging findings that acute alcohol use is associated with alterations in functional brain networks and the microstructural organisation of white matter in areas associated with emotive, cognitive, and introspective processes (Bjork & Gilman, 2014). Taken together, this suggests that reward and emotion are intertwined processes, which share common neural circuitry. That BD is primarily a disorder of mood, it is therefore no surprise that aberrant reward processes are often a feature of the disorder (Whitton et al., 2016).

6.4 Bipolar Disorder is Associated with Alterations to Reward and Affective Circuitry

The pathophysiology of BD is proposed to arise from dysfunction of prefrontal areas related to the cognitive control of emotion, and to disruptions in the ability of these areas to exert a top-down control over subcortical areas which are involved in the processing of affective stimuli (Phillips et al., 2008). Reduced connectivity within the medial PFC and the ventral anterior cingulate has been demonstrated in BD, this area is a critical region for emotion regulation through its afferent and efferent connections to frontal and subcortical areas (Anticevic et al., 2013). The results of this thesis supports these findings by demonstrating altered patterns of connectivity within an ECN containing these frontal cortical areas for participants with BD in comparison to control participants (Study Three). An internal regulatory loop containing ventro-medial areas of the ACC and the prefrontal cortex, where the paracingulate cortex resides, displays functional alterations in BD which is related to difficulties in adapting to changes in emotional and social contexts (Maletic & Raison, 2014). This loop mediates internally generated feeling states, such as feelings of sadness generated by memories (Maletic & Raison, 2014). In tandem the volitional cognitive control

network processes externally induced emotional states and ensures appropriate cognitive regulation of emotional responses (Maletic & Raison, 2014).

Many theories of the pathophysiology of BD contain reference to internal states and adaptive behaviours, introspective processes which are known to be altered within the disorder (Perry et al., 2018). This thesis has demonstrated increased functional activity of the cuneal cortex in a ventral DMN for BD participants in comparison to healthy controls (Study Three), this network supports the appraisal of internal stimuli and introspective processing. Moreover, the results of this thesis has demonstrated increased functional connectivity patterns within a dorsal DMN for BD participants in comparison to healthy controls (Study Three), this network functions to appraise and respond to internal and external emotional stimuli (Lindquist & Barrett, 2012). The smooth processing of internal autonomic and visceral stimuli, with corresponding neuronal responses in limbic circuitry is referred to as interoception (Craig, 2003). The processing of these visceral stimuli into predictive signals relies on higher order frontal regions involving the insula, ACC and OFC (Barrett & Simmons, 2015; Critchley et al., 2004; Friston et al., 2012), areas which are known to be altered within BD (Hibar et al., 2018). It is for these reasons that BD has been termed as "interoceptive psychosis", with the suggestion that fronto-parietal network dysfunction reflects the symptomatic expression of BD, leading to unstable and maladaptive internal representations of the external social world (Perry et al., 2018). However, the limitations of study designs to correlation rather than causation within cognitive neuroscience arrests the ability of the field to determine the mechanisms supporting these internal representations and the manner in which they influence affective and reward based behaviours. The findings of this thesis provide further support for this theory and identify network alterations of interoceptive and affective networks, independently and also in the presence of alcohol use.

Processing of risky choices in uncertain rewarding contexts in BD are not governed by a general tendency to risk-take but are informed by a reduced sensitivity to factors that promote and inhibit risky behaviour (Chandler et al., 2009). This may be reflected in reduced activation of the ACC during reward expectancy in those with BD, in comparison to healthy controls, where the reduced activation signals a disruption of affective based reward decisions (Chase et al., 2013). Moreover, top-down cognitive control of the ventral striatum (VS) is inhibited in rewarding contexts (Chase et al., 2013), as well as disturbed functional connectivity between the prefrontal cortex and the nucleus accumbens in BD (Trost et al., 2014). These alterations within the prefrontal and mesolimbic reward circuitry are suggested to reflect an overvaluation of outcomes that are strongly desired but suboptimal in the long term (Mason et al., 2014). Moreover, these alterations in reward and emotion circuitry are found to correspond with depression severity in participants with BD (Satterthwaite et al., 2015).

Differences between a diagnosis of unipolar depression and BD have been demonstrated during rewarding contexts, with BD participants demonstrating reduced activation of the NAcc, caudate, thalamus, putamen, and insula in comparison to depressed participants (Redlich et al., 2015). Across neuroimaging modalities, numerous studies have demonstrated distinct structural and functional alterations in reward/ affective processing circuits, between BD and unipolar depression, with findings demonstrated in connectivity and morphological differences in the DMN, fronto-parietal networks, dlPFC, ACC, parietal and temporal regions (Han et al., 2019). Moreover, large scale structural imaging studies in BD identify that the most robust findings of structural alterations in BD are found in regions corresponding to reward and emotion processing, such as inferior and middle frontal gyri, OFC, insula, and temporal areas (Hibar et al., 2018), as well as subcortically in the hippocampus (Hibar et al., 2016). Taken together these studies identify that neural circuitry

impacted in BD often corresponds to that used to process affective and reward processes, and that alterations correspond to mood alterations.

6.5 Alcohol Consumption in Bipolar Disorder is Associated with Compound alteration of Reward and Affective Circuitry

This thesis has demonstrated that areas of reward processing which have been associated with structural and functional abnormalities in BD are further impacted by alcohol use relative to psychiatrically healthy controls. For instance structural alterations in the cortical thickness of the ACC and particularly for BD participants the dlPFC (Study One), reduced connectivity strength of a subnetwork containing cortico-limbic and basal-ganglia nodes (Study Two) and increased connectivity of the paracingulate gyrus in an DMN and altered connectivity patterns within an ECN (Study Three). Taken together this suggests that pre-existing alterations to reward circuitry may be present in BD, which appear to be compounded by alcohol use, leading to additional aberrant reward and therefore we suggest affective processing in the disorder.

As previously identified the ACC and dlPFC support reward and emotion processing, these structures demonstrate lower cortical thickness in BD in comparison to control participants (Hibar et al., 2018) and independently are associated with lower cortical thickness in association with alcohol use (Mashhoon et al., 2014; Morris et al., 2019). The results of this thesis finds alterations in these structures for BD participants with the consumption of alcohol in a dose dependent manner (Study One) and suggest that this places the structures under compound stress which may lead to aberrant reward and affective processing, leading to mood lability in the disorder. Research demonstrating reductions in frontal lobe structures are frequently reported in BD and are associated with increased mood lability and poor illness trajectory (Bruder et al., 2017). Moreover, altered connectivity

between the ACC and dlPFC is suggested to related to difficulties in processing affective stimuli within the disorder (Jabbi et al., 2020). Additionally, within a sample of healthy participants, lower thickness of the dlPFC was predictive of increased alcohol use (Morris et al., 2019). The thesis also demonstrates an effect of alcohol on the topological configuration of an anatomical subnetwork involving the collective arrangement of nodes which support reward and affective processing for BD participants only (Study Two). Previous large-scale work based on the microstructural organisation of the fibre bundle has demonstrated widespread alterations to tracts involved in reward and emotion processing within the disorder, for instance the cingulum (Favre et al., 2019). Recent theoretical developments have conceptualised the brain as a complex network, with the potential to identify hierarchies of subnetworks which interact to give rise to complex cognitive processing and behaviour. This study (Two) identifies a specific subnetwork which is altered in the presence of alcohol and reflects areas which interact together and are vulnerable to compound alterations from BD and alcohol consumption. That many of the nodes contained within the subnetwork (ACC, OFC, caudate, hippocampus and temporal lobe) have been previously identified as independently impacted structurally or functionally by alcohol or BD, lends further credence to this theory.

Functional alterations within BD and in the presence of alcohol use have been identified within this thesis, however, Study Three remains the only manuscript to examine alterations to intrinsic functional networks in BD with alcohol consumption. These results of this study finds that there is an increase in connectivity of the paracingualte gyrus with a DMN, with a decrease in the connectivity patterns of an ECN for BD participants only. The cingulate gyrus is often implicated both structurally and functionally in cross sectional studies in the pathophysiology of BD (Hibar et al., 2018; Strakowski et al., 2012), it is also found to demonstrate progressive alterations in structure and function in longitudinal samples

(Lim et al., 2013). Moreover, alterations of the cingulate cortex appear in early onset BD and are associated with an insidious and malignant illness, additionally for all those with a diagnosis of BD changes in the cingulate across time are associated with clinical symptoms of the disorder, particularly depressive symptoms (Lim et al., 2013). The paracingualte gyrus demonstrates reduced cortical thickness in the disorder, which is not attributable to individual differences in cortical folding patterns (Fornito et al., 2008). Independently, alcohol use is associated with structural and functional alterations of the ACC for a range of alcohol consumptions and samples (Arienzo et al., 2020; Heikkinen et al., 2017; Hu et al., 2018; Mashhoon et al., 2014; Meda et al., 2017). Taken together, these previous studies support the findings of this thesis and provide support for functional alterations in BD in the presence of alcohol use. This demonstrated result provides plausible evidence that a key structure in reward and affective processes, nested within a cognitive affective network is altered in the disorder with alcohol use.

As previously discussed, alcohol is associated with increased dopamine availability within the mesocorticolimbic pathway, this originates in the ventral tegmental area (VTA) and projects to the VS, amygdala, and prefrontal cortex (Koob & Volkow, 2016). Dopamine neurons from the VTA project to the VS via direct and indirect pathways, the direct pathway mediates reward processing, while the indirect opposes adversive responses, thus maximal reward is obtained when both pathways are activated by alcohol (Volkow & Morales, 2015). The increase in dopamine in the neucleus accumbens is sufficiently large to induce associative learning, which increases the liklihood of further substance use through the association with pleasureable feelings, these conditioned responses are mediated by glutamatergic projections from the amygdala, hippocampus, and ventral PFC (Jacob & Wang, 2020). The development of compulsive alcohol use has been associated with the shift

of reward processing from the VS to the dorsal stritum an area assoicated with habit formation (Everitt & Robbins, 2005).

Alterations in reward expectancies and processing in BD is often attributed to a dysfunction in dopamine signalling, this is often particularly prevalent in (hypo)mania states with hyperdopaminergia thought to underlie impulsive and risk-taking behaviours (Whitton et al., 2016). However, treatment with a dopamine agonist is demonstrated to induce aberrant reward-related decision making in euthymic participants with BD, preference for high-risk, high-reward choices were demonstrated with no influence on mood symptoms (Burdick et al., 2014). Abnormal activity in the VS is found during reward anticipation, consumption and to predictive cues in BD, and may be reflective of alterations in dopamine transmission related to the disorder (Caseras et al., 2013; Mason et al., 2014; Nusslock et al., 2012) As noted, the effect of dopamine is mediated by glutamate signalling which originates in medial portions of the PFC, indivdiuals with BD have demonstrated increases in glutamate within the brain which may be associated with the demonstrated alterations in brain function during reward prediction (Gigante et al., 2012).

6.6 Strengths, Limitations and Future Directions

A number of methodological choices were made during the course of this thesis to improve measurement accuracy, reporting and replicability of the studies. For Study One all images were visually inspected for accurate boundary definition following parcellation by Freesurfer software to ensure correct parcellation of regions (Fischl, 2012). For Study Two, the pre-processing techniques employed allowed for the recreation of white matter trajectories using CSD tractography which can deconvolve multiple fibre tracts within a single voxel rather than the voxel wise averages obtained in diffusion tensor imaging (Tournier et al., 2008). For Study Three, data was decomposed functional networks using a

data-driven multivariate ICA approach (Calhoun et al., 2003) thus removing structural limitations placed by the researcher in seed-based approaches. Using this approach also provides superior power as ICA can identify weak signal contributions from a number of sources across the brain (Calhoun, 2018). The Committee for Best Practices in Data Analysis and Sharing (COBIDAS), arising from the Organisation of Human Brain mapping clearly outline the requirements for reporting of acquisition parameters, pre-precessing, statistical modelling, results, and reproducibility (Nichols et al., 2016). This thesis benefits from these methodological choices and reporting guidelines and ensures that our reporting is clear and robust, and our methods are rigorous and reproducible.

A strength of this thesis lies in the parcellation schemes used across the studies which provides a superior estimation of the anatomical description of cortical regions of interest, node definition, and areas of functional activation. There is no internationally agreed upon parcellation of the brain, which can make comparison between studies difficult as spatial scale can impact on results, particularly within connectome studies (Zalesky et al., 2010). For Study One and Two, parcellation was based on a probabilistic approach to region definition, using Freesurfer software which takes into account location, curvature and sulcal and gyral geometry of grey matter providing a subject-specific parcellation which increases anatomical sensitivity (Desikan et al., 2006; Fischl et al., 2002). For study Three, the Anatomy toolbox through SPM was used to identify anatomical reference points when interpreting functional activations. This software provides probabilistic anatomical labels based on microstructural analysis of the cortex in a sample of human *post-mortem* brains which reduces sources of error in using anatomically defined areas in functional data (Eickhoff et al., 2005).

A further strength of this thesis is the use of a data-driven multivariate fMRI data analysis approach (Study Three). The decomposition of resting state functional data into component maps is reliably reproduced across data sets and research groups (Laird et al.,

2011; Smith et al., 2009). These maps have the potential to identify biomarkers of pathology related to alcohol use or BD (Biswal et al., 2010) and the combined influence of alcohol in BD. This thesis represents a first endeavour to identify functional alterations for those with BD in the presence of alcohol use and may provide a proof of concept for the ability of fMRI to be used to develop treatments which are targeted towards the individual. Patterns of brain activity have been used in tandem with self-report mood scales to develop a spectral biomarker related to symptom severity in major depressive disorder (Scangos et al., 2021). This biomarker was then targeted with deep brain stimulation to normalise brain activity and alleviate the symptoms. This study identifies that patterns of brain activity have the potential to be used to identify pathology and develop personalised treatments related to mood disorders. Moreover, regional profiles of fMRI temporal features appear to provide reliable markers of individual differences thus further suggesting their utility in personalised medicine (J. Zhang et al., 2021; S. Zhang et al., 2021). Taken together, these studies and my results suggest that fMRI could be used to identify biomarkers and tailor treatments for people with a diagnosis of BD who consume alcohol.

This thesis was limited by the questionnaire used to quantify alcohol use: the AUDIT-C which is a validated and reliable questionnaire used in numerous clinical and research settings (Bradley et al., 2007; Bush et al., 1998; Higgins-Biddle & Babor, 2018; Kaarne et al., 2010). Despite the reliability of the instrument, participants are often not accurate historians and can misremember or alter their alcohol consumption to fall in line with social expectations of alcohol use (Halim et al., 2012). Therefore, the use of the questionnaire is limited by participant memory and social norms. However, the AUDIT-C remains one of the best tools to quantify alcohol use over the previous twelve months, and our scores are in line with another European sample (Bradley et al., 2007; Higgins-Biddle & Babor, 2018; Lange et al., 2016). While the AUDIT-C has been used to quantify alcohol use in a number of studies,

it is important to note that a standard drink differs from country to country, making comparison across studies challenging (Mongan & Long, 2015). This limitation has been overcome in some studies where longitudinal alcohol use reporting has been used to estimate the number of grams of alcohol consumed by participants on a weekly or monthly basis (Topiwala et al., 2017, 2021). However, while longitudinal alcohol scores provide a greater level of information for the researcher, in an Irish sample it has been demonstrated that participants who were asked to record their alcohol use daily were still underreporting their consumption (Long & Mongan, 2013). Despite these limitations and considerations, this thesis provides evidence that there was no difference in alcohol use between BD and control participants over a twelve month period.

A further limitation to the alcohol scores collected in this thesis, is that they were collected at one point and referred to alcohol use over the previous twelve months. This timeframe may not be indicative of the participants' alcohol use over their lifetime, and may reflect an under or over estimation of their consumption. However, an exclusion criteria for the larger study that this data was sourced from was the presence of a historical or current alcohol use disorder. This removed participants with higher levels of alcohol consumption across the previous twelve months and within their life time. The assessment of non-dependent alcohol use within a study population is difficult: blood measures are only sensitive to chronic alcohol use and direct metabolites of alcohol for instance, ethyl sulphate are insensitive to non-dependent alcohol use (Schröck et al., 2017; Paull et al., 2018). Is it likely that structural and functional alterations demonstrated within this thesis are associated with cumulative alcohol use over the years of alcohol consumption of the participants. Without detailed longitudinal information it is difficult to understand at what volume of alcohol use, number of years use or age of the participant this appears harmful to brain health. However, adolescent and young adult studies demonstrate harmful impacts of alcohol

use to brain health even at these younger ages (Galinowski et al., 2019; Ruan et al., 2019; Mashhoon et al., 2014). This suggests that alcohol as a toxic substance to the brain is harmful to younger brains. Moreover, recent large scale studies demonstrate alterations to numerous organs in association with low rages of alcohol use (Daviet et al., 2021; Evangelou et al., 2021; Topiwala et al., 2021). This points to the negative impact cumulative of low amounts of alcohol use across the lifespan. Additionally, a recent longitudinal study which measured the cumulative impact of alcohol use and brain included MRI at the end point demonstrated that even low levels of alcohol use were associated with structural alteration and poor cognitive outcomes (Topiwala et al., 2017). Taken together these studies demonstrate that whether alcohol use is measured longitudinally or cross sectionally, low levels of alcohol use are associated with harmful impacts to the brain. Moreover and importantly for people with a diagnosis of BD, clinically alcohol use is gathered as a self-report measure between the patient and clinician. This gives clinical relevance to the findings of this thesis and the numerous other studies that gather alcohol use via self-report.

A clear limitation of the study is the fact that we have not been able to replicate our findings within an independent sample. While our studies were adequately powered, the small effect sizes related to our findings, in particular Study One, suggests that alterations related to alcohol use may be subtle and require careful replication to confirm. Recent large-scale studies have demonstrated structural and functional alterations in association with alcohol use (Daviet et al., 2021; Evangelou et al., 2021; Topiwala et al., 2021) and therefore provide robust support for smaller scale work demonstrating an effect of alcohol use. However, others while demonstrating an effect of alcohol for all participants show no difference between controls and those with a diagnosis of BD or schizophrenia (Lange et al., 2016). The methods used in this thesis (Study One) were different in that we investigated cortical thickness in regions of interest, while Lange *et al.* (2016) investigated the whole

brain, despite this the discrepancies in findings support the need for replication of our work. The field of alcohol use research, particularly in regards to non-dependent use is limited; a lack of consistency in results highlights the need for replication to advance knowledge. Despite this limitation, a strength of this thesis is that we build on the neuroimaging modalities used across the studies, moving from a grey matter structural investigation, to a white matter structural network study, and then to a functional network study. This allows the thesis to identify structural alterations and relate them to demonstrated functional alterations, and demonstrate that there are key neural regions and networks which are impacted by alcohol in BD.

A further limitation are the methods used to measure and characterise alcohol use and functional connectivity: these processes have a dynamic flow, behaviour related to alcohol use changes across time, as does functional connectivity within the brain. I have measured functional connectivity using static functional activation, which is an average of activation in the brain across time and cannot describe the unfolding of dynamic phases or states (Cabral et al., 2017). Additionally, alcohol consumption has been measured at a single point estimate using the AUDIT-C, which is unable to capture changes in alcohol consumption dynamically. Therefore, a natural methodological extension to this thesis is to move from a static representation of functional activation within the brain to that of dynamic functional connectivity. Functional connectivity evolves over multiple timescales and is underpinned by the static structural network of the brain, structure does not define function but provides it with scaffolding for dynamic activity in changing contexts (Cabral et al., 2017; Sporns, 2012). Phase transitions may represent movement between cognitive states as the brain integrates cognitive processes and information as a function of changing internal and external stimuli (Shine et al., 2019). As alcohol consumption is an active process, it may be best understood through a dynamic lens to determine how the substance impacts network

repertoire and resultant cognitive processes and behaviours. Previous research has demonstrated that infusion of psilocybin modulates the repertoire and dynamic exploration of brain networks at rest (Lord et al., 2019), moreover, acute alcohol use is associated with static functional alteration in the resting brain (Bjork & Gilman, 2014). The use of application based mobile questionnaires may be able to record alcohol consumption in real time, in real life settings which have more ecological validity than a laboratory based questionnaire. Applying application based alcohol data, to dynamic models of functional connectivity may provide researchers with richer evidence of the impacts of alcohol use on functional architecture, reward and affective processes.

6.7 The Potential for this Thesis to Contribute to Irish Public Health Policy

As previously identified alcohol use is common in Irish society, with the average yearly consumption of a person aged over 15 estimated to be 10.8 litres of pure alcohol in 2019 (O'Dwyer et al., 2021). These figures are 19% higher than the Irish Government's aim of reducing per capita alcohol consumption to 9.1 litres by 2020 (Department of Health, 2018). The recently enacted Public Health (Alcohol) Act 2018 faced strong opposition from industry interest groups, its delay of three years represented the longest interval between publication and enactment of a law in Irish history (O'Dwyer et al., 2021). Historically the alcohol industry has had a nontrivial influence in alcohol policy in Ireland, this has been demonstrated through the successful campaign by the Vintners and the Irish Hotel Federation of Ireland in watering down lower blood alcohol content legislation in 1994 and the successful increase in the hours of alcohol availability in 2000 (Hope, 2006). The alcohol industry has increased its activities within the public health and academic medicine spheres under the guise of corporate social responsibility, however the reported results are often instrumental to the industries economic interests, for instance placing responsibility for

alcohol use on the individual and avoiding industry responsibility (Babor & Robaina, 2013). In an Irish context, the industry funded charity Drinkaware, was found to be less likely to tweet about alcohol and physical harms related to cancers, heart disease and pregnancy and was more likely to tweet about the behavioural aspects of alcohol use, in comparison with non-industry funded alcohol charities such as Alcohol Action Ireland (Hessari et al., 2019).

There is a distinct lack of awareness in the community of the link between alcohol use and health risks, despite the fact that alcohol is a leading cause of premature death and disability worldwide and plays a causal role in more than 60 health conditions (O'Dwyer et al., 2021; WHO, 2014). The Public Health (Alcohol) Act was to deliver on a number of key strategies such as minimum unit pricing for alcohol, health warnings on containers, structural separation of alcoholic drinks within shops, and advertising restrictions to address this lack of health-related knowledge and reduce consumption (Department of Health, 2019). That the Six Nations Rugby Championship in 2020 broadcast alcohol messaging every 15 seconds during matches, highlights the need for limits on alcohol advertising (Purves & Critchlow, 2021). Despite the push to enact the law a number of strategies have been slow to roll out, for instance minimum unit pricing and alcohol product labelling (Critchlow et al., 2021). Providing the Irish Government with robust and reliable evidence of the brain-based impact of alcohol use may advance the public appetite for public health reform and support a reduction in the influence of the alcohol industry on Irish health policy. Recent large-scale studies have demonstrated that even low levels of alcohol use are harmful to the brain and other organs, suggesting that the safer drinking guidelines set by government require reconsideration (Daviet et al., 2021; Evangelou et al., 2021; Topiwala et al., 2021). Replication of this thesis within a large-scale sample could provide further evidence of the extent of alcohol related brain-based harm to the individual and society as a whole, with an opportunity to relate this to the economic cost of alcohol related cognitive impairments,

dementias, hospitalisations, and impacts on mood and wellbeing. Moreover, this has the potential to increase the public's awareness of the physical impacts of alcohol use and broaden understanding to encompass effects on the brain. The results of this thesis and its potential replication would provide robust evidence to policy makers within the Department of Health, and also the Health Service Executive to inform future public health policy with a view to improving the health of all alcohol consumers in Ireland.

6.8 Thesis Conclusions

This thesis comes at a critical juncture socially for alcohol use more broadly in Ireland and for people with a diagnosis of BD. Alcohol is widely consumed in our community (O'Dwyer et al., 2021), with the majority of harms experienced by individuals who report non-dependent alcohol use (O'Dwyer et al., 2019). The majority of alcohol use research in BD is undertaken in samples of people who are comorbid for AUD and BD (Azorin et al., 2017; Nery et al., 2014), despite the fact that alcohol use is associated with mood lability and poor trajectory within the disorder (Goldstein et al., 2006; Gordon-smith et al., 2020). Independently, alcohol use and BD are associated with structural and functional alterations of circuitry related to emotion and reward (Maletic & Raison, 2014; Topiwala et al., 2021). This thesis conceptualised the brain as a network to understand the influence of alcohol on interacting regions of the brain for all participants and particularly for those with a diagnosis of BD.

Taken together, these three manuscripts identify over a range of neuroimaging modalities, that specific reward and affective networks as well as structures nested within them are compromised within the disorder in the presence of alcohol use. Alterations in reward expectances may impact a person with a diagnosis of BD negatively through the impact on neuroanatomy compromising reward and affective processing.

The thesis demonstrates that alcohol use is associated with alterations of cortical thickness in areas which support the top-down control of reward and affective processes. Moreover, for BD participants there are potentially compound alterations to the ACC and dlPFC in comparison to control participants, which may reflect aberrant processing of affective and rewarding stimuli, thus impacting on mood lability within the disorder. Alterations of cortical and subcortical regions related to reward, as well as aberrant reward

processing are reported to be features of the disorder (Hibar et al., 2016, 2018; Whitton et al., 2016). Additionally, previous work demonstrates global alterations in association with alcohol use to cortical thickness for control and BD participants, with no difference found between the groups (Lange et al., 2016), my results suggest that particular reward circuitry is additionally impacted within the disorder.

To advance on these findings, it was demonstrated that alcohol use is associated with increased connectivity for all participants in a subnetwork comprising fronto-limbic, basal ganglia and temporal nodes. Moreover, an additional subnetwork is found which is differently connected for participants with BD in comparison to healthy controls. This subnetwork is associated with reduced connectivity strengths of nodes involved in cortico-limbic and basal ganglia circuitry. These results point to a differential impact of alcohol use between control and BD participants to subnetwork topology. Previous structural connectivity work has demonstrated a disconnected subnetwork containing limbic and basal ganglia nodes, regions which support reward and emotion processes (Nabulsi et al., 2019). This thesis extends on that study and suggests that alcohol contributes to compound alterations in the disorder which may point to a neuroanatomical vulnerability to impact on reward and affective processing in BD.

Finally, the thesis uses resting state fMRI data to investigate the impact of alcohol use on intrinsic networks within the disorder. It is found that consuming alcohol is associated with altered patterns of connectivity within DMN and ECN networks. Additionally, having a diagnosis of BD and, moreover, having a diagnosis of BD and consuming alcohol is associated with aberrant functional connectivity within default mode and executive control networks. Aberrant introspective processes have been linked to an inability to disengage from emotional stimuli in BD and respond in a cognitively controlled manner to obtain relevant rewards (Perry et al., 2018). This manuscript advances our understanding of alcohol use on

intrinsic network connectivity and provides evidence that it is associated with aberrant function in networks supporting affective and introspective processes.

Together this body of work suggests that the methodological approach of network neuroscience can enhance our understanding of the brain-based impacts of alcohol use for all consumers and particularly for participants with a diagnosis of BD. The thesis identifies across a range of MRI modalities that there are common impacts both structurally and functionally to regions and circuitry subserving reward, affective and introspective processes. This thesis corroborates previous research that identifies BD as a mood disorder with aberrant reward processes and identifies the compound impact of alcohol on this neuroanatomy. Future examination of the dynamically reconfiguring of communication patterns in the presence of alcohol may further elucidate the impact of alcohol use in the disorder and its relation to mood lability and potential for poor clinical trajectory.

6.9 References

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