RESTING STATE FUNCTIONAL CONNECTIVITY AND ALCOHOL MISUSE

RESTING STATE FUNCTIONAL CONNECTIVITY AND ALCOHOL MISUSE IN HEAVY DRINKING ADULTS

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LAY ABSTRACT

Alcohol use disorder is a complex condition that is not yet fully understood, especially when it comes to the underlying changes in the brain. One theory suggests that individuals with mental health conditions show changes in one or more of three key networks in the brain. These networks are essential in understanding higher cognitive function and dysfunction (e.g., emotional regulation and problem solving). This theory suggests that the range of damage in these networks leads to the differences in symptoms and symptom severity that individuals experience. Prior research among alcohol users has found changes in connectivity in all three networks, suggesting they may all be involved. This study used neuroimaging to examine whether connectivity was associated with alcohol misuse. We found that connectivity in the network involved in decision-making and problem solving was associated with alcohol use severity, suggesting this network may be a useful target for new treatment strategies.

ABSTRACT

Purpose: The triple network model posits that the salience network (SN), default mode network (DMN), and central executive network (CEN), are essential in understanding higher cognitive function and dysfunction. These networks are affected variably across many psychiatric conditions. Previous functional magnetic resonance imaging (fMRI) research in alcohol use disorder (AUD) has found altered functional connectivity in the SN, DMN, and CEN, suggesting all three networks may be involved. The current study utilizes fMRI to investigate the association between resting-state functional connectivity in the SN, DMN, and CEN and alcohol misuse.

Methods: Fifty-two heavy drinking adults completed measures of alcohol use severity and quantity, as well as a 7-minute resting state scan.

Results: Linear regression was used to test if connectivity was associated with past 12-month AUD symptoms and number of heavy drinking days. Results revealed that CEN connectivity (right lateral prefrontal cortex seed co-activating with 19 clusters) was significantly associated with AUD symptoms (β = .425, p = .003), but not heavy drinking days. *Post-hoc* tests revealed six clusters co-activating with the CEN were associated with AUD symptoms – right middle frontal gyrus, right inferior parietal gyrus, left middle temporal gyrus, left cerebellum 7b, right cerebellum 7b, and left cerebellum 9. DMN and SN connectivity was not associated with drinking outcomes.

Conclusion: These findings illustrate that connectivity within the CEN, but not the DMN or SN, is associated with alcohol use severity. Regarding the triple network model, these results suggest that altered functional connectivity in heavy drinkers is located primarily in the CEN.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

AAL	Automated Anatomical Labelling
ACC	Anterior Cingulate Cortex
ADS	Alcohol Dependence Scale
AUD	Alcohol Use Disorder
AUDIT	Alcohol Use Disorder Identification Test
BOLD	Blood Oxygen Level Dependent
CEN	Central Executive Network
CONN	CONN Functional Connectivity Toolbox
DART	Diagnostic Assessment Research Tool
DICOM	Digital Imaging and Communication in Medicine
dlPFC	dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
FDR	False Discovery Rate
fMRI	functional Magnetic Resonance Imaging
FOV	Field of View
FWHM	Full-Width Half-Maximum
GE	General Electric
HiREB	Hamilton Integrated Research Ethics Boards
ICA	Independent Components Analysis
LP	Lateral Parietal
lPFC	lateral Prefrontal Cortex
MNI	Montreal Neurological Institute
mPFC	medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
NIFTI	Neuroimaging Informations Technology Initiative
PCA	Principal Components Analysis
PCC	Posterior Cingulate Cortex
PPC	Posterior Parietal Cortex
PTSD	Post-traumatic Stress Disorder
ROI	Region of Interest

rPFC	rostral Prefrontal Cortex
rs-fMRI	Resting State functional Magnetic Resonance Imaging
SMG	Supramarginal Gyrus
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences
T1	Longitudinal Relaxation Time
T2	Transverse Relaxation Time
TE	Echo Time
TI	Inversion Time
TLFB	Timeline Follow Back
TR	Repetition Time

DECLARATION OF ACADEMIC ACHEIVEMENT

All data presented was collected by Emma Marsden as part of a larger neuroimaging study conducted by Dr. Michael Amlung. Research questions were developed with aid from Dr. James MacKillop and Dr. Heather Moulden. Data analyses were performed by Emma Marsden in consultation with Tegan Hargreaves and Dr. James MacKillop. Data interpretation and write up of results was completed by Emma Marsden.

INTRODUCTION

Alcohol use contributes to 5.3% of all deaths globally, and yet, is a regular practice amongst adults around the world (Shield et al., 2020). In Canada, around 3.2% of individuals aged 15 or older met criteria for an alcohol use disorder (AUD) in the past 12-months (Pearson et al., 2013). Even higher were rates in the United States, where 5.3% of individuals aged 12 or older had an AUD in the past 12-months (Substance Abuse and Mental Health Services Administration, 2018). Moreover, alcohol use is associated with numerous physical and psychological consequences, such as cardiovascular diseases, major depressive disorder, and unintentional injuries (Grant et al., 2015; Knox et al., 2019; Shield et al., 2020).

In addition, alcohol use has been associated with considerable deficits in executive function. Executive functions are the higher-order cognitive processes, such as attentional shifting and working memory, that underly intentional and goal-directed behaviours (Suchy, 2009). Stephan et al. (2017) conducted a large-scale meta-analysis on the topic, including 77 studies examining executive functioning in individuals with a previous AUD and non-substance using controls. They found that executive function abilities were significantly affected by alcohol use across many neuropsychological tests of executive function, with moderate to large estimated effect sizes (Stephan et al., 2017). When examining executive function subcategories, planning and problem solving and flexibility and set shifting showed the largest effect sizes (*Hedges' g* = 0.773 and 0.663, respectively). These findings echo previous research and suggest that individuals with AUDs are very likely to demonstrate significantly poorer cognitive performance.

Although there is a breadth of research examining the cognitive consequences of alcohol use, the neurological underpinnings of the disorder are not yet fully understood. Neuroimaging techniques, such as resting state functional magnetic resonance imaging (rs-fMRI), are useful for exploring functional connectivity in the brain. Rather than examining changes in brain activation during a task or following a stimulus, rs-fMRI explores patterns of ongoing and synchronous activation at rest (Biswal et al., 1995). Task-related increases in brain activity are very small and represent a fraction of overall brain activity, compared to the high energy consumption of the resting brain (Fox & Greicius, 2010). Consequently, the signal-to-noise ratio of task-based approaches is inferior to that of rs-fMRI, with up to 80% of blood oxygen level-dependent (BOLD) modulation being discarded as noise in task-based approaches (Fox & Greicius, 2010). More generally, rs-fMRI avoids much of the cognitive and physical demand of task-based approaches and allows for greater inclusion of individuals.

One attempt to understand the psychopathology of AUD at the neurological level is through the triple network model. The triple network model posits that three interconnected networks, namely the salience network (SN), default mode network (DMN), and central executive network (CEN), are essential in understanding higher cognitive function and dysfunction (Menon, 2019; Menon, 2011). Together, these large-scale networks are important for self-referential mental processes such as detecting, filtering, and integrating sensory, affective, and cognitive information (SN), memory retrieval and emotional regulation (DMN), and complex problem solving and decision making (CEN; Menon, 2019; Menon, 2011). These networks are affected variably across many psychiatric conditions, including substance use disorders (Menon, 2019). The triple network model suggests that the specific networks affected and the range of

dysfunction in those networks leads to the presentation and severity of clinical symptoms. Therefore, the triple network model has the potential to explain the homo- and hetero-geneity of symptoms found in AUD at the disorder and individual level.

There is considerable research using rs-fMRI to investigate functional connectivity in alcohol users, however, the findings show minimal consistency. In an early study of individuals who reported binge drinking, rs-fMRI was used to analyze network connectivity strength (Weiland et al., 2014). Reduced network strength was found in the left executive control, sensorimotor, basal ganglia, and primary visual networks, relative to controls. As well, connectivity in the left executive control network was negatively associated with years of regular drinking, measured using a single self-report question, and alcohol problems, measured using the Alcohol Use Disorders Identification Test (AUDIT) and the Failed Control subscale of the Impaired Control Scale. Using a group-based design, Zhu et al. (2015) used rs-fMRI to investigate differences in connectivity between individuals with an AUD and controls with no current or past history of an AUD. Relative to controls, those with AUD showed increased connectivity within the salience, default mode, central executive, and prefrontal cortex networks. In addition, functional connectivity in these networks was significantly correlated with alcohol use severity, as measured by the Alcohol Dependence Scale (ADS). In a similar design, Vergara et al. (2017) examined patterns of functional connectivity between participants with suspected hazardous drinking patterns, determined by scores of 8 or greater on the AUDIT, and controls with no current abuse or dependence of alcohol. Compared to controls, those in the alcohol group displayed decreased connectivity among the salience, sensorimotor, visual, and precuneus networks, but increased connectivity between the reward system and areas of visual

processing. Between the sensorimotor and visual networks, there was a significant decrease in connectivity as AUDIT scores increased. A more recent study by Fede et al. (2019) used functional connectivity from participants with moderate to heavy alcohol use to predict alcohol use severity, as measured by the AUDIT. Neural data gathered using rs-fMRI was entered into a machine learning model, where features corresponding to between network connectivity with the salience, default mode, central executive, basal ganglia, visual, sensorimotor, auditory, and language networks, were strong contributors in predicting AUDIT score.

Other studies have examined rs-fMRI among participants using more than one substance. For example, Morris et al. (2022) compared functional connectivity between individuals using alcohol only, individuals using alcohol and one other substance, and individuals using alcohol and two or more additional substances. Those in the 3+ substance group showed decreased connectivity within the salience and temporal networks, compared to those in the mono or dual substance groups. When the groups were combined, connectivity in the SN and one of two clusters in the temporal network was significantly correlated with number of substances used and drug use severity, as measured by the Drug Use Disorder Identification Test. Overall, these studies suggest that individuals who consume alcohol at least regularly are characterized by differential patterns of functional connectivity in several different resting state networks. Moreover, there appears to be a strong link between functional connectivity and alcohol-related characteristics and outcomes (e.g., alcohol use severity, alcohol problems, drug use severity). With the triple network model in mind, these findings suggest there is increased DMN connectivity in alcohol users, and evidence for increased and decreased connectivity with the salience and executive control networks.

The aim of the current study was to use rs-fMRI to investigate whether patterns of functional connectivity could predict drinking outcomes in heavy drinking adults. We sought to examine three large-scale brain networks, namely the salience, default mode, and central executive networks. Previous studies examining these networks have produced inconsistent results, possibly due to the varying definitions of alcohol use (social drinkers, heavy drinkers, alcohol dependency, etc.) and the range of techniques used to analyze rs-fMRI. Based on the heterogenous findings in the literature and the limited use of the triple network framework in this population, we hypothesized that connectivity in the salience, default mode, and central executive networks would be associated with measures of alcohol misuse, but without greater specificity in terms of connectivity patterns. As well, we sought to explore specific clusters that may be driving the relationship between functional connectivity and drinking outcomes.

METHODS

Participants

Participants were recruited from the Hamilton, Ontario, Canada community. The inclusion criteria for the study were: 1) between the ages of 21-55, 2) right-handed, 3) fluent in the English language, 4) self-reported heavy drinking (on average > 14/7 drinks per week for males/females in the past three months), and 5) >1 self-reported heavy drinking episode weekly over the past three months (5+/4+ drinks for males/females within a single drinking episode). Participants were excluded from the study if they met any of the following criteria: 1) currently receiving or seeking treatment for alcohol related problems, 2) current Diagnostic and Statistical Manual of Mental Disorders, Fifth

Edition (DSM-5) substance use disorder other than alcohol or tobacco, 3) weekly or more frequent use of recreational drugs other than cannabis, 4) history of schizophrenia-spectrum disorders, psychotic disorders, bipolar disorder, or post-traumatic stress disorder (PTSD), 5) history of neurocognitive disorder or impairment, 6) magnetic resonance imaging (MRI) contraindications, 7) history of serious brain injury, 8) currently taking psychotropic medications or medications that could affect cerebral blood flow, 9) pregnancy, and 10) attending any study session with a positive breath alcohol concentration (BrAC > 0.00g%). Participants were also required to demonstrate cue reactivity (i.e., increased alcohol demand and craving) following a laboratory cue exposure to be considered eligible for the MRI scan. This requirement was related to a different aspect of the study, but ultimately determined who completed the MRI scan session. All inclusion and exclusion criteria, except for breath alcohol concentration, were assessed with an over the phone or online screening interview. Breath alcohol concentration was assessed at the beginning of each session using a commercial breathalyzer device. However, due to updated safety regulations following the COVID-19 pandemic, breathalyzer devices were no longer used, and sobriety was assessed using a self-report questionnaire. Therefore, for a subset of participants sobriety was assessed using the breathalyzer device and for the remaining participants the self-report questionnaire was used.

There were 110 participants enrolled into the study, with 52 completing all study sessions including the MRI scan.

Procedure

The study was approved by the Hamilton Integrated Research Ethics Board (HiREB; Project #7458). Individuals who met all eligibility criteria were enrolled in the study and scheduled for the first session. The first session was completed remotely via phone or zoom and consisted of a clinical interview assessing drinking behaviour and AUD symptoms, as well as a battery of questionnaires. After completion of the first session, participants received a $20 electronic gift card to a local store or online retailer.

Those who were interested in continuing were then scheduled for the second session. The second session was completed in-person at the Peter Boris Centre for Addictions Research at St. Joseph's Healthcare Hamilton, West 5th campus. During the session, participants completed several neurocognitive tasks and a standardized neutral and alcohol cue exposure protocol (Amlung & MacKillop, 2015; Amlung et al., 2012; MacKillop et al., 2011). In the neutral cue exposure, participants are seated at a small table in a neutral laboratory room and a glass of water is placed in front of them. Participants are then guided through a pre-recorded imagery script where they will periodically pick up the drink and take five deep breaths to inhale the smell of the drink. Following the recording, participants complete post-cue assessments measuring alcohol craving, alcohol demand and subjective affect. After the neutral cue exposure is complete, participants are escorted to a simulated bar environment for the alcohol cue exposure. The procedures were identical to the neutral cue exposure (e.g., listening to the recording, smelling the drink, and post-cue assessments); however, the beverage was a standard sized drink of the participants preferred alcohol beverage. Participants were instructed not to drink the neutral or alcohol beverage under any circumstances. Following the cue exposure protocol, responses on the neutral and alcohol post-cue

assessments were reviewed to determine eligibility for the MRI scan session. Participants were required to show an increase in at least one index of alcohol demand (e.g., intensity, breakpoint, Omax[1]) and at least one craving index (e.g., "How much do you want a drink of alcohol?", "How high is your urge for a drink of alcohol?") to be considered eligible for the MRI scan session. This concluded the second session and participants received another $20 electronic gift card to a local store or online retailer.

Those who were eligible and interested in completing the study were scheduled for the third and final session including an MRI at the Imaging Research Centre at St. Joseph's Healthcare Hamilton. The MRI scan lasted roughly one hour and was administered by an MRI technologist. Throughout the MRI, participants completed an anatomical scan, resting-state scan, viewed images of neutral and alcohol beverages, and completed four runs of the Alcohol Purchase Task. When the scan was completed, participants were debriefed about the purpose of the study and given a $40 gift card to a local store or online retailer.

Measures

Participants completed a variety of questionnaires and tasks throughout the study. However, a subset of these measures assessing demographics and alcohol use were included in the present analyses. The included measures were administered on the computer or in a virtual one-on-one meeting with a trained researcher.

[1] Alcohol demand is assessed using the Alcohol Purchase Task, where individuals make choices about how many alcoholic drinks they would consume at a range of prices (hypothetically). Intensity represents alcohol consumption at free price. Breakpoint represents the price that suppresses alcohol consumption to zero. Omax represents an individual's maximum expenditure.

Demographics. Participants provided information on their age, sex assigned at birth, gender identity, race, ethnicity, marital status, employment status, student status, years of education, income, weight, height, and handedness.

Diagnostic Assessment Research Tool (DART). Diagnosis of current alcohol use disorder was obtained using the DART for DSM-5 (Schneider et al., 2022). Total symptoms endorsed on the DART was a primary dependent variable, defined as a continuous variable.

Timeline Follow Back (TLFB). Participants reported their daily alcohol consumption for the thirty days prior to assessment (Sobell & Sobell, 1992). Indices on this measure include total number of drinks consumed, drinks consumed per week, and number of heavy drinking days (5+/4+ drinks for males/females on a single day). Number of heavy drinking days was a primary dependent variable.

Alcohol Use Disorders Identification Test (AUDIT). The AUDIT is a 10-item self-report measure assessing alcohol-related problems (Saunders et al., 1993). Total scores on the measure range from 0-40. A score of 8 or greater typically reflects hazardous or harmful alcohol consumption.

Imaging Acquisition

The scanner used was a 3-Tesla General Electric (GE) Discovery scanner. Images were gathered using a 32-channel receive-only radio frequency head coil and a

transmit radio frequency body coil. High-resolution longitudinal relaxation time (T1)-weighted structural images were acquired using a 3D inversion-recovery gradient-echo sequence [GE 3D Brain Volume Imaging (BRAVO); inversion time (TI) = 450 ms, echo time (TE) = 3.1 ms, repetition time (TR) = 7.4 ms, flip angle = 12°, 256 x 256 matrix, field of view (FOV) = 25.6 cm]. These images were used as an anatomical reference. Additionally, a resting state scan was collected lasting 7-minutes. During the resting state scan, participants were told to lay still, keep their eyes open, clear their mind, and try not to think about anything. However, participants were allowed to blink. Resting state images were gathered using a transverse relaxation time (T2*)-interleaved echo-planar imaging sequence with 40 axial slices acquired (TE = 30 ms, TR = 2000 ms, flip angle = 90°, 3.5 mm thick, 64 × 64 matrix, FOV = 22.4 cm).

Data Processing and Data Analysis

Using Statistical Parametric Mapping Software, version 12 (SPM; http://www.fil.ion.ucl.ac.uk/spm) raw MRI data were converted from Digital Imaging and Communication in Medicine (DICOM) file format to Neuroimaging Informations Technology Initiative (NIFTI) file format for analyses. Structural and functional images were preprocessed using the default processing pipeline for volume-based analyses in CONN Functional Connectivity Toolbox, version 19.b (CONN; www.nitrc.org/projects/conn; Whitfield-Gabrieli & Nieto-Castanon, 2012). Steps for structural scans included centering to (0, 0, 0) coordinates (translation), segmentation into grey matter, white matter, and cerebrospinal fluid, and normalization to Montreal Neurological Institute (MNI) space.

Preprocessing steps for functional scans included realignment and unwarping (subject motion estimation and correction), centering to (0,0,0) coordinates (translation), outlier detection [artifact detection tools (ART)-based identification of outliers], segmentation into grey matter, white matter, and cerebrospinal fluid, normalization to Montreal Neurological Institute (MNI) space and spatial smoothing [4.5-mm full width at half maximum (FWHM) Gaussian filter]. For outlier identification, the intermediate motion correction setting was used; subject motion greater than 0.9mm or signal changes greater than 5 standard deviations were identified and removed.

Following the preprocessing of functional scans, analyses were conducted in CONN using the weighted General Linear Model analysis type. Region of interest (ROI)-to-ROI and seed-to-voxel analyses were performed. Realignment, quality control, and scrubbing were included as first level covariates. Using CONN's predefined list of seeds, we selected all seeds which were part of the SN [anterior cingulate cortex (ACC), left anterior insula, right anterior insula, left rostral prefrontal cortex (rPFC), right rPFC, left supramarginal gyrus (SMG), right SMG], DMN [medial prefrontal cortex (mPFC), left lateral parietal (LP), right LP, posterior cingulate cortex (PCC)], and CEN [left lateral prefrontal cortex (lPFC), right lPFC, left posterior parietal cortex (PPC), right PPC]. A voxel-level false discovery rate (FDR) corrected threshold of $p < 0.00001$ and cluster-level FDR corrected threshold of $p < 0.001$ was used to identify clusters that were significantly correlated with the seed regions. Finally, fisher r-z transformed functional connectivity values for each subject, from each cluster showing significant and positive connectivity with the specified seed, were extracted, and imported into Statistical Package for the Social Sciences, version 28 (SPSS; https://www.ibm.com/analytics/spss-statistics-software) for further statistical analyses.

Clusters showing negative connectivity with seeds were not included in these analyses. Clusters for each seed were combined using principal component analysis (PCA), extracting the first principal component for each seed. The direct oblimin method with the default delta of zero was used for oblique rotation.

Principal components and drinking indices (e.g., drinks per week, number of AUD symptoms, etc.) were entered into a correlation matrix to determine the seed regions for each network of interest. Seeds used in these analyses were selected based on a significant correlation with the drinking index. If there were no seeds in a network of interest significantly correlated with the drinking index, the seed with the largest correlation coefficient was selected. Similarly, if multiple seeds in a single network were correlated with the same drinking index, the seed with the largest correlation coefficient was selected. The selected seeds and drinking variables were then analyzed using linear regression. Each regression model included functional connectivity (i.e., principal component representing connectivity with the seed) as an independent variable and a drinking index as a dependent variable. Covariates included in the regression models were sex and education.

Post-hoc testing was conducted at the cluster-level for principal components that were significantly associated with drinking indices. Clusters were labelled using the automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Each cluster was analyzed using linear regression. Data were examined for missingness and outliers. Outliers were identified (Z scores > 4.0) on the total drinks over 30-days and drinks per week indices of the TLFB. The indices were log-transformed for all data analyses. There were no completeness or data quality issues on any other measures.

RESULTS

Sample Characteristics

Participants include 52 heavy drinking adults recruited from the Hamilton, Ontario community. Demographic variables examined included sex assigned at birth, age, race, and education. Drinking characteristics including drinks per week, drinks per 30-days, heavy drinking days per 30-days, AUDIT score, AUD symptoms over the past 12 months, and AUD+ status were also examined. Results are presented in Table 1.

Differences in demographic and drinking characteristics between males and females were examined; see Table 2. Education (p = .007) differed significantly between male and female participants. Females had approximately 5 years of post-secondary education compared to approximately 3.5 years of post-secondary education for males. Education and sex were included as covariates in the primary analyses. No other demographic variables differed between males and females. Regarding drinking characteristics, drinks per week (p = .003), drinks per 30-days (p = .003), AUDIT score (p = .009), and AUD+ status (p = .031) differed significantly between males and females. Males were characterized by greater alcohol consumption and severity.

Preliminary Analyses

Fifteen unique principal components were created. All principal components had an Eigenvalue of greater than 1 and accounted for between 20-53% of the total variance; see Table 3.

Of the networks of interest, only the CEN demonstrated significant correlations between seeds and drinking indices. There were no seeds in the DMN or SN that were correlated with the drinking indices. Significant correlations included the left lPFC, right

lPFC, and right PPC with past 12-month AUD symptoms and the right lPFC with heavy drinking days. The largest correlation coefficients in the DMN were between the left LP and past 12-month AUD symptoms and the mPFC and heavy drinking days. The largest correlation coefficients in the SN were between the left SMG and past 12-month AUD symptoms and the left SMG and heavy drinking days. See Table 4 for full results.

Seeds included in the analyses with past 12-month AUD symptoms as the dependent variable were the left SMG (-60, -39, 31; SN; depicted in Figure 1a), left LP (-39, -77, 33; DMN; depicted in Figure 1b), and right lPFC (41, 38, 30; CEN; depicted in Figure 1d). In the analyses with heavy drinking days as the dependent variable, seeds were the left SMG (SN; depicted in Figure 1a), mPFC (1, 55, -3; DMN; depicted in Figure 1c), and right lPFC (CEN; depicted in Figure 1d).

Primary Network-level Findings and Past 12-Month AUD Symptoms

The regression models including DMN connectivity (left LP co-activating with 16 clusters) and SN connectivity (left SMG seed co-activating with 12 clusters) were not significant and connectivity was not associated with past 12-month AUD symptoms. The regression model including CEN connectivity (right lPFC seed co-activating with 19 clusters) was significant [$F(3, 48) = 4.083$, $p = .012$, $R^2 = .203$], with connectivity being significantly associated with past 12-month AUD symptoms ($\beta = .425$, $p = .003$). See table 5 for full results.

Exploratory linear regressions were carried out to further investigate which of the clusters co-activating with the right lPFC were significantly associated with AUD symptoms. We found that six clusters were associated with past 12-month AUD

symptoms – one in the orbital part of the right middle frontal gyrus (42, 52, -6; β = .319, *p* = .034; depicted in Figure 2a), one in the right inferior parietal gyrus (48, -46, 46; β = .296, *p* = .041; depicted in Figure 2b), one in the left middle temporal gyrus (-66, -50, -6; β = .307, *p* = .031; depicted in Figure 2c), one in the left cerebellum 7b (-40, -64, -48; β = .308, *p* = .040; depicted in Figure 2d), one in the right cerebellum 7b (34, -64, -46; β = .312, *p* = .028; depicted in Figure 2e), and one in the left cerebellum 9 (-6, -60, -62; β = .372, *p* = .007; depicted in Figure 2f). See table 6 for full results.

Primary Network-level Findings and Heavy Drinking Days

The regression model including SN connectivity (left SMG seed co-activating with 12 clusters) was not significant, and connectivity was not significantly associated with number of heavy drinking days. Although the regression models including DMN connectivity [mPFC co-activating with 17 clusters; $F(3, 48) = 3.328$, $p = .027$, $R^2 = .172$] and CEN connectivity [$F(3, 48) = 3.924$, $p = .014$, $R^2 = .197$] were significant, connectivity was not significantly associated with number of heavy drinking days in either network. See table 7 for full results.

DISCUSSION

The present study aimed to investigate whether patterns of rs-fMRI could predict alcohol use quantity and severity in adults reporting heavy drinking. Specifically, functional connectivity in the networks corresponding to the triple network model: the salience, default mode, and central executive network. Although our hypotheses were somewhat exploratory, our results were in partial support of our primary predictions. We

found that positive CEN connectivity was associated with alcohol use severity, but default mode and salience network connectivity was not.

At a more granular level, we found that functional connectivity in the CEN (right lPFC) was significantly associated with past 12-month AUD symptoms. *Post-hoc* analyses revealed that these findings were driven primarily by connectivity with the right middle frontal gyrus, right inferior parietal gyrus, left middle temporal gyrus, left cerebellum 7b, right cerebellum 7b, and left cerebellum 9. Surprisingly, functional connectivity within the CEN was not associated with number of heavy drinking days.

Our finding of an association between CEN connectivity and past 12-month AUD symptoms is consistent with our hypothesis. Moreover, our finding is in line with previous work by Zhu and colleagues (2015) showing that increased connectivity within the CEN was correlated with alcohol use severity. The CEN, otherwise known as the task-positive network, is involved in working memory, problem solving, and goal-directed decision making (V. Menon, 2011). Individuals with an AUD demonstrate significant deficits in these processes (see review, Stephan et al., 2017), likely due to underlying deficits in the CEN. Therefore, the association between positive connectivity and past 12-month AUD symptoms may reflect neural compensation. Alcohol consumption has been associated with reductions in gray matter and alterations in white matter microstructure (Daviet et al., 2022; Yang et al., 2016). Further, a study using task-based fMRI found reduced white matter integrity was associated with increased functional connectivity in the dorsolateral prefrontal cortex (dlPFC) in individuals with AUDs and individuals reporting problematic drinking (Jansen et al., 2015). This increased recruitment of a prefrontal area in individuals with white matter damage suggests the brain is trying to compensate. Therefore, the association between positive functional

connectivity in the CEN and AUD symptoms, in the current study, may represent a compensatory mechanism.

A deeper exploration into CEN connectivity revealed six clusters associated with past 12-months AUD symptoms. The middle frontal gyrus, a suspected node of the executive control network, has been proposed to be involved in reorienting attention from internal to external stimuli (Di & Biswal, 2014; Japee et al., 2015; Zhu et al., 2015). During task-based fMRI paradigms, individuals with an AUD have shown both activation and deactivation in the right middle frontal gyrus, relative to controls (for meta-analysis, see Quaglieri et al., 2020). Based on the function of the middle frontal gyrus and its role in a task-positive network, connectivity in this region at rest may reflect the neural compensation hypothesis mentioned prior. The inferior parietal gyrus, including the supramarginal and angular gyri, is involved in phonological short-term memory and solving mathematical problems (Mahmood et al., 2013). A study in adolescents with high frequency substance use found that increased activation of the left angular and supramarginal gyri during the go/no-go task predicted substance use in the following 18 months (Mahmood et al., 2013). Although comparisons between task-based and resting state fMRI should be interpreted with caution, our findings complement the prior results and suggest the inferior parietal gyrus may be associated with current alcohol use. The middle temporal gyrus has been consistently associated with the understanding of communication through words and gestures (Papeo et al., 2019). During a task-based fMRI paradigm, individuals with an AUD showed increased activation in the left middle temporal gyrus, compared to controls (Parks et al., 2010). Our findings build on the prior results by suggesting that the middle temporal gyrus also shows positive connectivity at rest. As a whole, the cerebellum plays a role in emotion integration and coordination.

Although the cerebellum is not commonly associated with alcohol use, Manzardo et al. (2005) found that in infants, developmental markers of cerebellar functioning (e.g., age of walking, muscle tone at 5 days of life) predict alcohol use at 30 years of age. More research is needed to clarify how connectivity in the cerebellum is related to alcohol use in adulthood. Taken together, functional connectivity in relation to these six brain regions may be important targets for future research in alcohol users.

Functional connectivity in the default mode and salience networks was not associated with drinking outcomes. This finding was inconsistent with our hypothesis and prior research. One study found that increased connectivity within the default mode and salience networks predicted alcohol severity among individuals with an AUD (Zhu et al., 2015). As well, among moderate to heavy alcohol users, features corresponding to connectivity with the default mode and salience networks predicted alcohol severity (Fede et al., 2019). However, key differences exist between the methods in these prior studies and the current study. First, compared to the seed-based approach used in the current study, prior research used independent components analysis (ICA) to analyze rs-fMRI data, potentially leading to inconsistencies in the association of a node with a network. Second, the previous studies focused on within-network (Zhu et al., 2015) and between-network (Fede et al., 2019) functional connectivity, whereas both within- and between-network connectivity is included here. It's possible that the more focused approach used in prior studies may not be comparable to the exploratory approach used currently. Third, the relationship between functional connectivity and drinking variables were assessed using different methods: correlation (Zhu et al., 2015), machine learning analysis (Fede et al., 2019), and in the current study, regression. Findings from various statistical methods are useful for interpreting overall trends in the literature but are

difficult to compare directly due to fundamental differences. Fourth, alcohol use severity was assessed using the ADS (Zhu et al., 2015), AUDIT (Fede et al., 2019), and presently, the DART. These measures differ in their clinical utility and may be assessing unique facets of problematic alcohol use. It is possible that one or more of these methodological differences can account for the varying findings.

Interestingly, functional connectivity in the networks of interest was not associated with number of heavy drinking days. There are two potential explanations for these findings. Few studies have examined functional connectivity as a predictor of alcohol consumption (e.g., quantity and frequency). Of those studies, to our knowledge, none have investigated functional connectivity in the salience, default mode, and central executive networks. Hence, these findings are novel, and replication is warranted. That in mind, our findings suggest that positive connectivity in the networks of interest may not be associated with heavy drinking patterns. In addition, the triple network model posits that alterations in functional connectivity leads to the range and severity of symptoms experienced. Therefore, it's possible that connectivity in the salience, default mode, and central executive networks can better predict clinical drinking outcomes, such as those assessed by the DART or AUDIT, than patterns of alcohol consumption (e.g., quantity and frequency).

There are several limitations of the study which should be considered. First, our sample size was relatively small and may have limited our statistical power. However, this did not lead us to use a more liberal statistical threshold and keeps our statistical approach in line with current recommendations (Yeung, 2018). Second, the sex ratio in our sample was unequal, with more females than males included. Although this has historically been a concern, a recent review suggests that findings around sex and/or

gender differences in functional connectivity are inconsistent, inconclusive, and do not warrant claims that the brain is sexually-dimorphic (Eliot et al., 2021). Therefore, we predict that any impact of sex will be minimal. Nevertheless, the impact of gender should be explored in future studies with diverse demographics given patterns of altered functional connectivity associated with sexual and/or gender minority identities (Nicholson et al., 2022; Uribe et al., 2021). Third, the data in these analyses were drawn from a larger MRI study which did not include a non-substance using control group, making comparisons between alcohol users and non-users not possible. Fourth, the current analyses of rs-fMRI included only positive functional connectivity. It is possible that by not including negative functional connectivity, potential associations between decreased connectivity and drinking outcomes are being overlooked. Finally, by using PCA to combine all the clusters co-activating with a seed into one variable, we are exploring overall functional connectivity, rather than examining connectivity within and between the network independently.

In conclusion, our study provides evidence of a positive relationship between functional connectivity in the CEN and AUD symptoms in adult heavy drinkers. On the other hand, we did not find evidence of a positive relationship between positive functional connectivity in the default mode and salience networks and alcohol use severity (e.g., past 12-month AUD symptoms) or patterns of alcohol consumption (e.g., heavy drinking days). Regarding the triple network model, these results suggest that in heavy drinkers, altered functional connectivity is located primarily in the CEN, rather than the default mode or salience networks. Additional research is needed to explore how results may differ when examining within- and between-network connectivity separately. As well, future studies may benefit from examining decreased functional

connectivity within the networks of interest. Ultimately, these findings offer an initial examination of the triple network model in heavy alcohol users. We offer a foundation for future research analyzing the association between neuro-functional activity and clinical symptoms, which may inform novel therapeutic targets for intervention.

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APPENDIX A – Figures and Tables

Table 1. Sample Characteristics

	Mean (*SD*); n (%)
Sex (Male)	20 (38.5%)
Age	34.73 (8.56)
Race (White)	49 (94.2%)
Education	16.56 (2.08)
Drinks/Week	20.13 (13.51)
Drinks/30-days	86.25 (57.89)
Heavy Drinking Days	9.40 (7.24)
AUDIT	12.12 (5.72)
Past 12-Month AUD Sx	3.21 (2.92)
AUD+	32 (61.5%)
Mild	12 (37.5%)
Moderate	7 (21.9%)
Severe	13 (40.6%)

Note. AUD disorder severity was determined based on number of symptoms endorsed on the DART: 2-3 = mild, 4-5 = moderate, 6-11 = severe

Table 2. Sex Differences in Sample Characteristics

	Males ***n* = 20**	**Females** ***n* = 32**		
	Mean (*SD*); *n* (%)		***t*/*x*²**	***p***
Age	36.90 (9.89)	33.38 (7.46)	1.46	.150
Race (White)	19 (95.0%)	30 (93.8%)	0.04	.851
Education	15.60 (2.01)	17.16 (1.92)	-2.79	**.007**
Drinks/Week	27.05 (17.67)	15.80 (7.66)	3.17	**.003**
Drinks/30-days	115.89 (75.74)	67.72 (32.82)	3.17	**.003**
Heavy Drinking Days	10.80 (8.18)	8.53 (6.57)	1.10	.276
AUDIT	14.70 (6.22)	10.50 (4.80)	2.74	**.009**
Past 12-Month AUD Sx	3.80 (2.91)	2.84 (2.91)	1.15	.254
AUD+	16 (80%)	16 (50%)	4.68	**.031**
Mild	7 (43.8%)	5 (31.3%)	0.53	.465
Moderate	2 (12.5%)	5 (31.3%)	1.65	.200
Severe	7 (43.8%)	6 (37.5%)	0.13	.719

Note. AUD disorder severity was determined based on number of symptoms endorsed on the DART: 2-3 = mild, 4-5 = moderate, 6-11 = severe

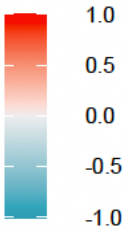
Table 3. Eigenvalues and Percent of Variance Accounted for by Principal Components

Component	Eigenvalue	Total Percent of Variance
SN: ACC	4.511	28.193%
SN: left Anterior Insula	4.060	40.597%
SN: right Anterior Insula	4.343	28.952%
SN: left rPFC	4.646	30.970%
SN: right rPFC	4.926	27.369%
SN: left SMG	6.309	52.578%
SN: right SMG	4.352	27.197%
DMN: mPFC	5.086	29.919%
DMN: left LP	4.741	29.634%
DMN: right LP	2.804	28.037%
DMN: PCC	3.556	22.226%
CEN: left lPFC	3.966	22.034%
CEN: left PPC	5.005	33.366%
CEN: right lPFC	4.513	23.753%
CEN: right PCC	3.782	22.247%

Table 4. Correlation Matrix

Correlations between principal components (connectivity with seed regions), demographic variables and alcohol indices.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	--																					
2	.43	--																				
3	.49	.39	--																			
4	.24	.24	.68	--																		
5	.15	.01	.20	.17	--																	
6	.13	.06	.26	.20	.67	--																
7	.28	.14	.34	.27	.60	.74	--															
8	.22	-.03	-.03	.15	.48	.59	.41	--														
9	.14	.02	.00	.09	.49	.48	.49	.60	--													
10	.14	.32	.09	-.04	.22	.41	.20	.34	.32	--												
11	.24	.22	.21	.10	.60	.62	.58	.43	.44	.59	--											
12	.09	.24	.25	.19	-.08	.11	.08	-.05	.07	.29	-.02	--										
13	.41	.12	.46	.37	.07	.04	.14	-.01	-.04	.00	.04	.43	--									
14	.16	.16	.28	.22	-.17	-.01	.00	-.11	.06	.09	-.17	.67	.40	--								
15	.26	.19	.41	.39	.04	.18	.17	.02	.18	.22	.00	.60	.59	.72	--							
16	.15	-.02	-.06	-.10	-.09	-.15	-.09	-.07	.08	.23	.08	.06	.09	-.09	.00	--						
17	-.05	-.07	.05	.17	-.19	-.13	-.16	-.02	-.25	-.11	-.19	.04	.24	.09	.12	-.20	--					
18	-.11	-.05	.14	.08	-.07	-.04	.03	-.08	-.13	-.25	-.20	-.07	.17	-.28	-.15	-.11	.37	--				
19	.14	.23	.12	.01	-.09	.12	.04	-.10	-.02	.16	-.01	.30	.12	.41	.32	-.06	-.16	-.18	--			
20	.18	.10	-.04	-.26	-.02	.09	.10	-.08	.01	.10	.01	.18	.11	.27	.21	.27	-.30	-.23	.76	--		
21	.27	.22	.16	.06	-.01	.04	.18	-.09	.07	.06	.17	.06	.07	.19	.11	.23	-.42	-.30	.52	.58	--	
22	.23	.20	.05	.03	-.09	-.06	.11	.06	.21	.19	.14	.17	.05	.33	.20	.15	-.15	-.37	.44	.47	.80	--



Note. Colours reflect effect size (r); 1 = mPFC (DMN), 2 = left LP (DMN), 3 = right LP (DMN), 4 = PCC (DMN), 5 = ACC (SN), 6 = left anterior insula (SN), 7 = right anterior insula (SN), 8 = left rPFC (SN), 9 = right rPFC (SN), 10 = left SMG (SN), 11 = right SMG (SN), 12 = left lPFC (CEN), 13 = left PPC (CEN), 14 = right lPFC (CEN), 15 = right PPC (CEN), 16 = Age, 17 = Sex, 18 = Education, 19 = Past 12-month AUD Symptoms, 20 = AUD Diagnostic Status, 21 = Total Drinks During a 30-day Period, 22 = Number of Heavy Drinking Days

Table 5. Linear Regression Results for Past 12-Month AUD Symptoms

Variable	B	SE B	β	*t*	*p*	R^2
Past 12-Month AUD Sx						.057
Sex	-.627	.896	-.106	-.700	.487	
Education	-.163	.217	-.116	-.750	.457	
SN: left SMG	.348	.422	.119	.823	.414	
Past 12-Month AUD Sx						.092
Sex	-.563	.880	-.095	-.640	.525	
Education	-.194	.208	-.138	-.935	.355	
DMN: left LP	.644	.403	.221	1.601	.116	
Past 12-Month AUD Sx						.203
Sex	-1.195	.842	-.201	-1.418	.163	
Education	.012	.206	.009	.060	.952	
CEN: right lPFC	1.242	.401	.425	3.098	.003	

df = 3, 48

Table 6. Linear Regression Results for Right lPFC Clusters and Past 12-Month AUD Symptoms

Variable	B	SE B	β	*t*	*p*	R^2
Past 12-Month AUD Sx						.131
Sex	-.638	.860	-.107	-.742	.461	
Education	-.033	.217	-.023	-.152	.880	
Right middle frontal gyrus, orbital part	13.558	6.195	.319	2.189	.034	
Past 12-Month AUD Sx						.125
Sex	-.715	.864	-.120	-.828	.412	
Education	-.084	.211	-.060	-.395	.695	
Right inferior parietal gyrus	7.674	3.646	.296	2.105	.041	
Past 12-Month AUD Sx						.133
Sex	-.714	.859	-.120	-.831	.410	
Education	-.093	.209	-.066	-.444	.659	
Left middle temporal gyrus	10.207	4.601	.307	2.219	.031	
Past 12-Month AUD Sx						.126
Sex	-.954	.875	-.161	-1.090	.281	
Education	-.028	.220	-.020	-.125	.901	
Left cerebellum 7b	9.937	4.699	.308	2.115	.040	
Past 12-Month AUD Sx						.136
Sex	-1.089	.880	-.183	-1.238	.222	
Education	-.181	.203	-.129	-.893	.376	
Right cerebellum 7b	6.814	3.014	.312	2.261	.028	
Past 12-Month AUD Sx						.182
Sex	-.693	.834	-.117	-.830	.410	
Education	-.225	.197	-.160	-1.139	.260	
Left cerebellum 9	7.440	2.616	.372	2.844	.007	
Past 12-Month AUD Sx						.095
Sex	-.899	.892	-.151	-1.009	.318	
Education	-.169	.208	-.120	-.810	.422	
Left middle frontal gyrus	8.514	5.199	.229	1.638	.108	
Past 12-Month AUD Sx						.102
Sex	-.971	.894	-.163	-1.086	.283	
Education	-.124	.211	-.088	-.585	.561	
Left inferior parietal gyrus	5.546	3.124	.250	1.765	.084	
Past 12-Month AUD Sx						.082
Sex	-.874	.899	-.147	-.972	.336	
Education	-.113	.218	-.081	-.519	.606	
Right middle temporal gyrus	5.027	3.584	.204	1.403	.167	
Past 12-Month AUD Sx						.014
Sex	-.406	.910	-.068	-.446	.658	
Education	-.161	.213	-.115	-.757	.453	
Right insula	4.860	4.070	.176	1.194	.238	

Past 12-Month AUD Sx						.084
Sex	-.738	.885	-.124	-.834	.408	
Education	-.260	.212	-.185	-1.227	.226	
Left lingual gyrus	-6.338	4.371	-.206	-1.450	.154	
Past 12-Month AUD Sx						.085
Sex	-.428	.894	-.072	-.478	.635	
Education	-.208	.208	-.148	-1.000	.323	
Right caudate nucleus	5.776	3.964	.204	1.457	.152	
Past 12-Month AUD Sx						.050
Sex	-.723	.911	-.122	-.793	.432	
Education	-.175	.218	-.125	-.801	.427	
Right median cingulate and paracingulate gyrus	1.615	2.906	.081	.556	.581	
Past 12-Month AUD Sx						.073
Sex	-.821	.900	-.138	-.913	.366	
Education	-.180	.210	-.129	-.857	.395	
Left insula	4.125	3.351	.173	1.231	.224	
Past 12-Month AUD Sx						.060
Sex	-.759	.904	-.128	-.839	.405	
Education	-.168	.215	-.120	-.781	.439	
Right precuneus	2.878	3.233	.127	.890	.378	
Past 12-Month AUD Sx						.045
Sex	-.657	.904	-.111	-.727	.471	
Education	-.201	.213	-.143	-.945	.349	
Right inferior occipital gyrus	-.881	3.509	-.035	-.251	.803	
Past 12-Month AUD Sx						.066
Sex	-.841	.911	-.142	-.923	.360	
Education	-.179	.212	-.127	-.844	.403	
Right cerebellum crus 2	3.098	2.909	.152	1.065	.292	
Past 12-Month AUD Sx						.044
Sex	-.633	.906	-.106	-.698	.488	
Education	-.205	.213	-.146	-.958	.343	
Right inferior temporal gyrus	-.237	2.805	-.012	-.084	.933	
Past 12-Month AUD Sx						.052
Sex	-.710	.905	-.119	-.784	.437	
Education	-.187	.213	-.134	-.878	.384	
Right superior frontal gyrus, medial orbital part	-1.737	2.755	-.090	-.631	.531	

df = 3, 48

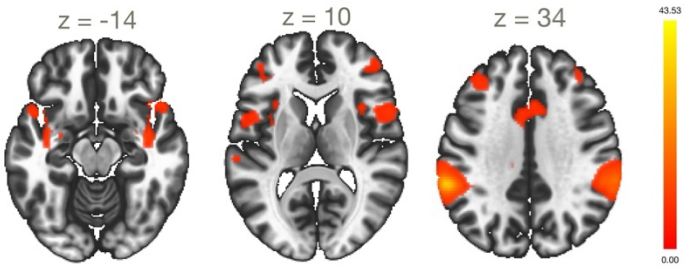
Table 7. Linear Regression Results for Heavy Drinking Days

Variable	B	SE B	β	*t*	*p*	R^2
Heavy Drinking Days						.146
Sex	-.284	2.114	-.019	-.135	.894	
Education	-1.174	.512	-.337	-2.292	.026	
SN: left SMG	.719	.997	.099	.721	.474	
Heavy Drinking Days						.172
Sex	-.299	2.081	-.020	-.144	.886	
Education	-1.184	.494	-.340	-2.398	.020	
DMN: mPFC	1.371	.957	.189	1.433	.158	
Heavy Drinking Days						.197
Sex	-1.157	2.097	-.079	-.552	.584	
Education	-.929	.514	-.267	-1.808	.077	
CEN: right lPFC	1.893	.998	.262	1.897	.064	

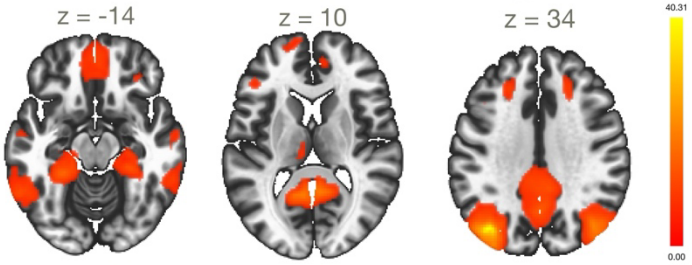
df = 3, 48

Figure 1a-d. Axial views of the salience, default mode, and central executive network examined in the current study

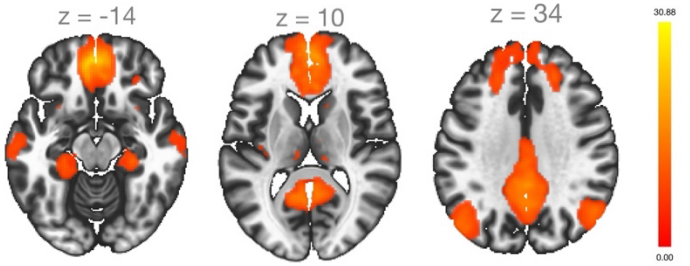
a) Salience Network



b) Default Mode Network (left lateral parietal)



c) Default Mode Network (medial prefrontal cortex)



d) Central Executive Network

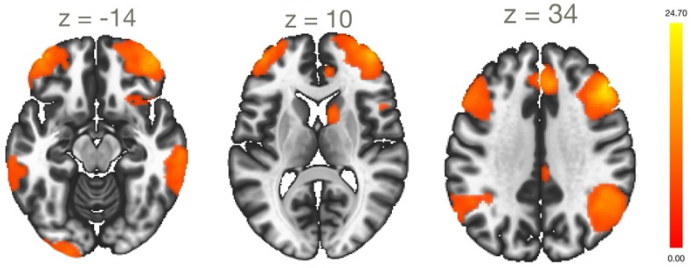
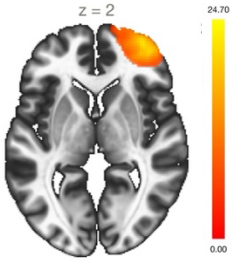
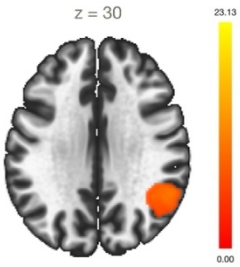


Figure 2a-f. Axial views of the clusters in the central executive network significantly associated with AUD symptoms

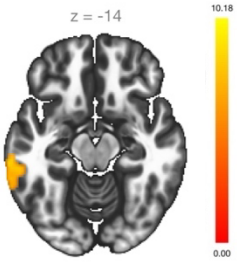
a) *Right middle frontal gyrus, orbital part*



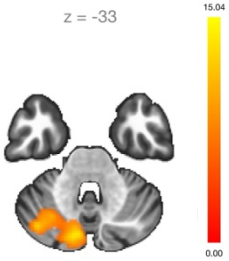
b) *Right inferior parietal gyrus*



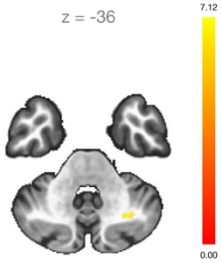
c) *Left middle temporal gyrus*



d) *Left cerebellum 7b*



e) *Right cerebellum 7b*



f) *Left cerebellum 9*