Mesial prefrontal cortex and alcohol misuse: dissociating cross-sectional and longitudinal relationships in UK Biobank

Ying Zhao, PhD[1]; Nikolina Skandali, MD, PhD[1]; Richard A.I. Bethlehem, PhD[1]; Valerie Voon, MD, PhD[1,2]

Affiliations:

1: Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom

2: Institute of Science and Technology for Brain-Inspired Intelligence, Fudan University, Shanghai, China

Corresponding to:

Ying Zhao, Department of Psychiatry, University of Cambridge, Level E4, Addenbrooke's Hospital, Cambridge CB2 0QQ United Kingdom. Phone: +44-7721877883. Email: yz617@cam.ac.uk

Valerie Voon, Department of Psychiatry, University of Cambridge, Level E4, Addenbrooke's Hospital, Cambridge CB2 0QQ United Kingdom. Email: vv247@cam.ac.uk

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Abstract

Background: Alcohol misuse is a major global public health issue. The disorder is characterized by aberrant neural networks interacting with environment and genetics. Dissecting the neural substrates and functional networks that relate to longitudinal changes in alcohol use from those that relate to alcohol misuse cross-sectionally is important to elucidate therapeutic approaches.

Methods: To assess how neuroimaging data, including T1, resting-state fMRI, and diffusion-weighted imaging, relate to alcohol misuse cross-sectionally and longitudinally in UK Biobank, the present study analyzed the population-based normative sample with range of alcohol misuse, ages 45 to 81 years old with 24,784 participants in cross-sectional analysis and 3,070 in longitudinal analysis 2 years later.

Results: Cross-sectional analysis shows alcohol use is associated with a reduction in dorsal anterior cingulate cortex (dACC) and dorsomedial prefrontal (dmPFC) grey matter concentration and functional resting-state connectivity (nodal degree: T=-12.99, p<10^-17). Reduced dACC/dmPFC functional connections to the ventrolateral prefrontal, amygdala, striatum relate to greater alcohol use. In longitudinal analysis, higher resting-state nodal degree (T=-3.27, p=0.0011) and T1 grey matter concentration in the ventromedial prefrontal (vmPFC) cortex relate to reduced alcohol intake frequency 2 years later. Higher vmPFC and frontal-parietal executive network functional connectivity associate with lower subsequent drinking longitudinally.

Conclusion: Dorsal versus ventromedial prefrontal regions are differentially related to alcohol misuse cross-sectionally or longitudinally in a large UK Biobank normative

dataset. Our study provides a comprehensive understanding of the neurobiological substrates of alcohol use as a state or prospectively, thereby providing potential targets for clinical treatment.

Introduction

Alcohol misuse/abuse is a major global public health issue. Alcohol use disorders (AUD) are among the most prevalent mental disorders worldwide (1, 2). In the United Kingdom (UK) and globally, alcohol misuse is the greatest risk factor for death, ill-health and disability amongst 15-49 years old with UK societal costs of £21 to £52 billion (3). AUD has been associated with aberrant neurocircuitry interacting with environmental, social and genetic influences (4, 5). The neural substrates mapping to the cognitive processes underlying core addiction theoretical mechanisms of incentive salience, negative emotionality and the transition to compulsive behaviors have been systematically reviewed in the Addictions Neuroclinical Imaging Assessment (ANIA) (4, 5), in which extensive cortical and subcortical brain structures and networks were found relevant to alcohol misuse.

Whilst important to identify the neural mechanism and negative impacts of alcohol use, a more important target is to identify biomarkers that relate to change or successful reduction of alcohol use and therefore, facilitate developing theoretical support for clinical interventions. In the recent decade, more efforts have been given to identify such biomarkers with task-based functional MRI (tfMRI) and grey matter volume/concentration associating with prospective alcohol use initiation or change (6-8). For example, in a recent large-scale analysis (N=2,423), smaller dorsolateral and insular grey matter volume was found to be significantly related to the predisposition and initiation of alcohol use spanning across adolescent to middle-age adults (6). In an adolescent study, grey matter volume and activation of the bilateral superior frontal gyrus and right precentral gyrus in tfMRI at the 14-years old associated with alcohol misuse at the age 16 (7). Similar adolescent studies identified reduced cortical thickness

and/or activation of rostral anterior cingulate and right superior frontal and frontal pole in 12- to 14-year olds relating to moderate to heavy drinking by age 18 (8). Activation of prefrontal regions relating to alcohol use change was also frequently reported in other tfMRI studies with relevant alcohol-related cognitive processes such as response inhibition (9-11) and alcohol cue reactivity (12), albeit with smaller sample sizes. In summary, previous studies point towards a reduced frontal centered structural and functional network relating to executive, control, and inhibitory function, which is engaged in alcohol misuse initiation and/or change longitudinally.

Based on previous findings, we hypothesize that the brain networks that relate to prospective longitudinal changes in alcohol use can be dissected from those related to alcohol use as a state in the frontal networks engaging cognitive control and inhibitory processes. We address the hypothesis within the UK biobank data, a normative population with a range of alcohol consumption patterns. The Biobank brain imaging dataset contains nearly 40,000 participants aged 45 to 81 years old scanned with multimodal magnetic resonance imaging (MRI), including T1-weighted structural data, resting-state functional MRI (rfMRI), and diffusion-weighted imaging (DWI) (13). The extensively validated Alcohol Use Disorders Identification Test (AUDIT) (14) was assessed at baseline and is a sensitive index of problem alcohol misuse. Longitudinally, alcohol intake frequency was assessed in a large subset (more than 3,000) of participants. Thus, the Biobank dataset offers a unique combination of cross-sectional and longitudinal data, to identify vulnerable neural substrates and functional network-based biomarkers.

Here we analyzed the Biobank dataset to first ask how brain structure and functional connectivity in an adult normative population relate to alcohol use in cross-sectional

analysis and more importantly, associate with alcohol use perspective changes in longitudinal analysis (see Figure 1. analytic flowchart). We use a network approach, investigating the resting-state functional connectivity networks, over and above the grey and white matter structure, so that we can identify biomarkers based on the connectivity and functional hemodynamics. The hemodynamic-based biomarker is potentially more useful as a target for brain stimulation (15). In addition, we also systematically estimate the convergence evidence between resting-state fMRI (rfMRI) and structural MRI. To our knowledge, our study is the first large sample, multimodal MRI particularly functional connectivity-based, prospective study for alcohol use.

Methods and Materials

Participants

Imaging data from 39,679 participants from the UK Biobank (project ID 64044) were filtered for alcohol use, smoking, age, gender, total intracranial volume, and data quality in T1, rfMRI, and DWI. Demographics and usable participant numbers are reported in Table S1 and Table S2.

Behaviors

Participants' alcohol use scores are based on the Alcohol Use Disorders Identification Test (14) (AUDIT) with alcohol intake frequency used for longitudinal change (reversing the original coding so that higher coding correspond to more frequent intake. We coded: 6=daily drinking; 5=3-4 times/week; 4=1-2 times/week; 3=1-3 times/month; 2=special occasion; 1=never. Thus, alcohol intake frequency data are in the same direction as

AUDIT). We add participants' smoking data into the analysis as a comparison with alcohol. Smoking is based on pack-years (daily cigarettes smoked number for longitudinal change).

Age, gender, imaging data collection site, and total intracranial volume were included as control variables. Other potentially confounding variables, including depression, anxiety, Body Mass Index (BMI), and blood pressure were assessed and included in the Supplementary materials (Table S1, Figure S11).

Brain imaging data processing

The preprocessing steps are described in the Biobank documentation (https://biobank.ctsu.ox.ac.uk/crystal/ukb/docs/brain_mri.pdf). Based on the preprocessed data, we calculated voxelwise grey matter concentration (from T1), nodal degree and functional connectivity (FC) (from rfMRI), and FA (from DWI). More details are given in Supplementary materials.

General linear models relating brain measures to behaviors

The general linear models were built on each voxel with all available participants for each imaging modality and behaviors of interest.

For simple linear models, we first regressed out age, gender, total intracranial volume, and data collection site from the voxel signal intensity, e.g., voxel grey matter concentration, nodal degree, or functional connectivity. Then we related the residual values to the AUDIT scores (alcohol intake frequency change for longitudinal analysis).

The simple linear models were built with Matlab (R2020b) 'fitlm' function and effect size (Cohen's f^2) was reported. Multiple linear models are given in details in the Supplementary materials.

We further built five interaction models between AUDIT scores and age/gender, pack-years and age/gender, AUDIT and pack-years, using Matlab 'fitlm' with 'interaction' terms.

We conducted similar analyses for smoking, as a comparison with alcohol, to demonstrate the potential common effects of substance misuse. Aging was analyzed in the cross-sectional analysis to show the normal aging brain pattern as a comparison with alcohol use and smoking.

Multiple comparisons corrections are given in Supplementary materials. Brain statistical results are mapped onto brain maps using Matlab package DPABI (16) and BrainNet Viewer (17).

Support vector regression models

Given the advantage of the Biobank's large sample, we built support vector regression (SVR) models on the cross-sectional grey matter and rfMRI nodal degree data. The SVR model with split-half cross-validation is used to test if the brain-behavior relationships are stable and replicable. The SVR models were built with the 'fitrlinear' function in Matlab with the 'svm' learner. We use a brain atlas with 625 similar sized ROIs respecting the region boundaries of the automated anatomical labeling (AAL) 90 atlas (18). Pearson

correlation between predicted and real behavioral residual scores was used to measure the SVR split-half cross-validation model accuracy. We give an example of the SVR model settings in the Supplementary materials.

Results

Our analytic flow chart is shown in Figure 1. We report the behavioral scores and their relationships with T1 grey matter concentration, rfMRI nodal degree and FC. DWI FA results are reported in Supplementary materials. We focus on the simple linear regression results between variables of interest and brain measure residual scores, controlling for age, gender, intracranial volume, and data collection site from grey matter signal intensity. The model accuracy of the SVR was reported following the univariate regression analyses in each section.

The multiple linear regression, where all variables were analyzed in one regression model, demonstrated the same pattern of results as the simple linear regression. The interaction models did not show significant interaction effects, which means alcohol use (smoking) and age contributed to brain functional and structural changes independently. Alcohol use (smoking) has no interaction with gender on the brain measures.

Behavioral scores

The behavioral data from the timepoint 1 alcohol (N=24,784, male participants N=11,156) T1 VBM analysis is reported (Figure S1 in the Supplementary materials) (age in mean (standard deviation): 63.46 (7.47) years old; AUDIT scores, 5.19 (4.16)). In the

smoking analyses, there were N=30,778 participants (pack-years 5.08 (11.69)); and N=8,157 smokers only (19.17 (15.67)). The Pearson correlation between AUDIT scores and alcohol intake frequency at the 1st timepoint is r=0.58 (p<10^-17, N=24,631).

We analyzed longitudinal alcohol intake frequency change from the 2nd versus 1st imaging visits (N=3,070). The year difference between 2nd and 1st imaging visit is an average of 2.35 years (standard deviation: 0.71, range: 1-7 years, 71% with 2 years difference and 26% with 3 years difference). The majority (68%) participants drinking frequency were unchanged, 18% reduced (negative scores), while 13% increased (positive scores). For the frequent daily drinkers (code 6, N=457), 27% (N=124) reduced drinking.

Cross-sectional grey matter voxel-based morphometry

Alcohol use

Greater alcohol use was related to reduced grey matter concentration in dorsal anterior cingulate/dorsomedial prefrontal cortex (dACC/dmPFC) (Bonferroni corrected voxel p<0.001, cluster size>400 mm^3; peak coordinates: 0,41,29; T=-7.37, p=10^-13, effect size=0.0022; cluster size=916 mm^3) (Figure 2). Same grey matter correlates can be seen when related to 1st timepoint alcohol intake frequency (Figure S2).

The peak cluster can also be seen in the SVR model (Figure 2) (model accuracy r=0.12 (range:0.05~0.15, 10,000 times cross-validation; p<0.00001, permutation test 10,000 times).

Smoking

Greater smoking (in number of packs per year) was also related to reduced grey matter

concentration in dACC/dmPFC, thalamus, caudate, and bilateral insula (Bonferroni corrected voxel p<0.001, cluster size>400 mm^3) (Figure 2). The prefrontal cluster includes dACC/dmPFC, which overlaps with alcohol use grey matter correlates (peak coordinates: 0,42,27; T=-13.45, p<10^-17, effect size=0.0059; cluster size=177,346 mm^3).

The above map was similar when only including smokers. Due to the non-normal distribution of smoking pack-years, we only included smokers in the SVR analysis (Figure 2) (model accuracy r=0.16 (range:0.05~0.21, 10,000 times cross-validation; p<0.00001, permutation test 10,000 times). The dACC/dmPFC region is significant in both simple linear regression and SVR.

The effects of age and grey matter are reported in Supplementary materials.

Cross-sectional resting-state fMRI nodal degree

Voxel nodal degree reflects connectivity of a voxel to other voxels, assessed as the total number (weight) of connections of a given voxel with all other voxels, that passed a connectivity threshold (Pearson correlation r>0.2). With resting fMRI, we first analyzed how nodal degree relates to alcohol use, smoking, and aging, to understand how these variables related to brain function from a network perspective.

Alcohol use

Greater alcohol use was related to reduced nodal degree in whole brain grey matter. The peak region (at threshold T<-10, cluster size>400 mm^3) is in dACC/dmPFC (peak coordinates: 0,40,24, T=-12.99, p<10^-17, effect size=0.0069, cluster size=8,288 mm^3)

(Figure 3). The finding converged with alcohol use grey matter correlates including when analyzed controlling for grey matter concentration. Same nodal degree brain correlates can be seen when related to 1st timepoint alcohol intake frequency (Figure S2). To note, large extent regions are significant (Figure S8-9) with more liberal T values. We focus on dACC/dmPFC as it is the peak cluster in both T1 and rfMRI results.

The dACC/dmPFC cluster can also been seen in the SVR model (Figure 3), model accuracy r=0.11 (range:0.07~0.14 in 10,000 times cross-validation; p<0.00001, permutation test 10,000 times).

Smoking

Greater smoking pack-years was related to reduced nodal degree in dACC/dmPFC and bilateral superior and middle frontal gyri (T<-10, cluster size>400 mm^3) (Figure 3). The dACC/dmPFC cluster is significantly related to smoking (peak coordinates: 2,40,26, T=-11.38, p<10^-17, effect size=0.0043, cluster size=1,104 mm^3) and was evident when controlling for alcohol use and grey matter concentration.

The above map is similar when only including smokers. The dACC/dmPFC and bilateral middle and superior frontal gyri are significant in both simple linear regression and SVR (model accuracy r=0.13; range:0.08~0.18 in 10,000 times cross-validation; p<0.00001, permutation test 10,000 times).

The effects of age and nodal degree are reported in Supplementary materials.

Cross-sectional resting-state functional connectivity

Given the dACC/dmPFC nodal degree overlap with alcohol use, smoking, and age, we further conducted dACC/dmPFC functional connectivity (FC) analyses to investigate dissociable patterns of connectivity. The dACC/dmPFC peak cluster from nodal degree ($T<-11$), which overlaps with alcohol grey matter VBM (Bonferroni corrected voxel $p<0.001$), was used as a region of interest (ROI) in the seed-based whole-brain FC analysis.

Alcohol use and smoking

Both greater alcohol use and smoking were associated with reduced FC between dACC/dmPFC and other brain regions with no increased FC observed (Figure 4; significant region reports in Supplementary materials Table S3 for alcohol use and Table S4 for smoking). Reduced connectivity with bilateral inferior frontal, dorsolateral prefrontal and lateral orbitofrontal cortices was specific to alcohol use and unrelated to smoking or aging, whereas reduced connectivity with bilateral superior frontal gyri and medial orbitofrontal cortices was specific to smoking. In contrast, dACC/dmPFC decreased subcortical connectivity with putamen, caudate, thalamus and amygdala were observed for both alcohol and smoking but unrelated to aging highlighting common subcortical effects across substance misuse.

The FA results reported in Supplementary materials show negative correlations for both alcohol and smoking in white matter tracts surrounding dACC/dmPFC including the corpus callosum and cingulum.

Relating brain measures to longitudinal behavioral change

We repeated the multimodal brain – behavior univariate analyses utilizing the longitudinal behavioral data, including alcohol intake frequency change and daily smoking change. We used scores in the second imaging visit minus the scores in the first and related these to brain measures from the first imaging visit investigating biomarkers of perspective behavioral change. We found nodal degree and grey matter concentration significantly related to alcohol intake frequency change, but not smoking change.

With all available participants, we found greater nodal degree (resting-state) and grey matter concentration (T1) was related to reduced drinking frequency at approximately 2 year follow-up (Figure 5). The ventromedial prefrontal cortex (vmPFC) in particular was significant in both resting-state nodal degree and T1 grey matter analyses (nodal degree, GRF corrected voxel $p<0.01$ and cluster $p<0.05$, convergent regional peak coordinates: -4,56,-20, T=-3.27, p=0.0011, effect size=0.0035, cluster size=1,376 mm^3; T1, GRF corrected voxel $p<0.02$ and cluster $p<0.05$, convergent regional peak coordinates: -1,39,-29, T=-3.24, p=0.0012, effect size=0.0034, cluster size=1,445 mm^3). In addition, ventral striatum and amygdala also showed significance in the grey matter analysis.

We further separated the participants into six groups based on their drinking frequency in the first imaging visit. Then we related brain nodal degree to the drinking frequency change and only consider participants who changed. The frequent drinkers (group 6) drove the nodal-degree relationship in the vmPFC region (GRF corrected voxel $p<0.01$ and cluster $p<0.05$, Figure 5) which converges with the whole group nodal-degree findings (convergent region peak coordinates: 10,48,-22, T=-3.06, p=0.0027, effect size=0.0768, cluster size=1,224 mm^3). Greater nodal degree of bilateral subcortical regions in caudate, putamen, pallidum and thalamus also related to lower subsequent

alcohol consumption (left hemisphere peak coordinates: -8,-26,12, T=-3.47, p=0.0007, effect size=0.0988, cluster size=4,672 mm^3; right hemisphere peak coordinates: 22,14,-4, T=-3.41, p=0.0009, effect size=0.0951, cluster size=5,440 mm^3).

Since vmPFC had a convergent significant effect on alcohol use change, we further analyzed which functional connections from vmPFC relate to alcohol use change (Figure 5). Greater vmPFC connectivity to bilateral frontal-parietal central executive network (19) related to subsequent lower alcohol use change (GRF corrected voxel $p<0.01$ and cluster $p<0.05$). Regions and statistics are reported in Supplementary materials.

Discussion

We highlight a multimodal dissociation of structural and functional brain networks in cross-sectional and longitudinal relationships with alcohol use in a large normative older adult cohort. The dACC/dmPFC region cross-sectionally relates to the alcohol misuse (in past one year measured by AUDIT) and smoking severity (measured by pack-years) convergently across grey matter concentration, rfMRI nodal degree and FC, and white matter fractional anisotropy using both linear regression and machine learning methods. Smoking severity shows a similar but independent effect in the dACC/dmPFC but with differing functional connectivity patterns. Both forms of substance use appear to show similar effects as aging in this region but also show effects independent of aging. In contrast, ventral and subcortical regions such as the vmPFC, ventral striatal, and amygdala integrity across both grey matter concentration and nodal degree, and its connectivity with the fronto-parietal central executive network relates to resilience and vulnerability specifically in alcohol use frequency in two years. Thus, we highlight

dissociable roles of the mesial prefrontal cortex with alcohol misuse on dorsal structures of dmPFC/dACC implicated in impaired top-down cognitive control; in contrast, we highlight ventral and subcortical structures of the vmPFC, ventral striatum, and amygdala, in relating to prospective resilience and vulnerability implicated in incentive motivation, negative emotionality, and habit theories of addictions.

Cross-sectional effect of dACC/dmPFC

The dACC/dmPFC region appears to relate to the cumulative effects of substance misuse. Within grey matter, smoking seems to have a stronger effect than alcohol use, which might be because pack-years considers lifetime nicotine use, while the AUDIT only considers the past one year. The dACC is activated in both alcohol cue reactivity and craving behaviors (20). This hub region is implicated in cognitive interference and control tasks in healthy controls and other substance misuse, such as opiate (21) and cocaine (22). dACC glutamate is further negatively correlated with alcohol use symptom severity (23) and is reduced in opiate-dependent subjects (21), along with impairments in neuronal density and functional viability (24) in substance misuse.

Similar to our present findings, previous studies also show general whole brain grey matter and white matter reductions in alcohol misuse (25-28). In Biobank, as the dataset has increase from ~10,000 to ~25,000, while general whole brain reduction was reported relate to alcohol consumption with replication (29, 30), the dACC/dmPFC can also be seen with the peak reductions. The dACC is closely located beside the ventricles and enlarged ventricles with atrophy in surrounding brain structures are seen in alcohol misuse (31). As alcohol use has a similar whole brain atrophy pattern as aging effects (32, 33), alcohol adding extra structural atrophy over and above aging effects may be

implicated (28, 34, 35). Critically, we show that alcohol use and aging are independently related to the reduction in grey matter, white matter, nodal degree, and functional connectivity.

Interestingly, although dACC/dmPFC show convergent effects in alcohol use, smoking, and aging, the FC patterns show distinct patterns. Decreased dACC/dmPFC and striatal FC was observed across both alcohol and smoking reported previously in resting fMRI in nicotine addiction (36) and in response inhibition task-based fMRI in alcohol misuse (37). In heavy alcohol users, our findings that negative relationships in FC between dACC and dorsolateral and ventrolateral prefrontal regions are consistent with impairments observed in executive functioning in addictions such as working memory, planning, attentional set shifting, and inhibitory processes (38-40). The unique FC with amygdala is consistent with the role of negative emotional theories in alcohol addictions (41-43). In contrast, dACC FC in smoking participants shows lower connectivity with superior prefrontal cortex, a region implicated in the development of alcohol misuse in adolescents (7).

The peak of white matter FA reduction was seen in the genu and body of the corpus callosum and cingulum for alcohol use, smoking, and aging, which converge with the T1 and resting-state findings. In a longitudinal study in relapsed alcohol users, white matter in the callosal genu and body showed accelerated decline (27) whereas abstinence shows the capacity to improve white matter integrity (27). Decreased FA in white matter tracts such as the internal capsule may represent the structural basis of the functional connectivity relationships observed in fronto-striatal substrates.

Prospective alcohol resilience: vmPFC and its functional connectivity

Greater vmPFC, ventral striatal, and amygdala structural and/or functional integrity related to alcohol use resilience in 2 years. The opposing interpretation similarly may be applied of lower structural and functional integrity relating to greater subsequent alcohol use. The vmPFC finding converges with observations of impaired vmPFC state-prediction error activity in goal-directed control predicting alcohol relapse behaviors (44). In an addiction and obesity study, the vmPFC activity was anticorrelated with the return of appetitive conditioned responding (45). Our finding of lower FC between vmPFC and the fronto-parietal network supports this relationship with impairments in representation of goal-directed control and executive impairments (46-48). Previous studies have found reduced grey matter concentration/volume in the frontal executive control network are risk factors for alcohol misuse (6-8) and our study supports the prospective resilience role of the control network in alcohol use from the connectivity-based analysis. Interestingly, a recent co-twin control study suggested the alcohol use can result in reduced cortical thickness in the frontal cognitive control and salience network by comparing alcohol use twins with co-twins who drank less (49). Longitudinal studies in adolescent and young adult have reported extensive evidence that lower frontal volume related to predisposition towards alcohol misuse (50). Using an alcohol-related Pavlovian-to-instrumental transfer paradigm, the activation of medial frontal related to the relapse of use in young adults (51). Our study suggested that the reverse direction that higher connectivity between vmPFC and the executive control network associates with reduction in alcohol use in older adults is also plausible, indicating potentially modifiable brain networks for clinical intervention.

The limbic substrates' relationship with subsequent behaviors highlights the critical role of incentive motivation and negative emotionality theories and extinction processes in alcohol misuse (5). Reward-related ventral striatal activity and threat-related amygdala activity are correlated with drinking initiation in the first stage of alcohol addiction (52). Increased dopamine and opioid transmission in basal ganglia and extended amygdala are a well-documented finding in driving the rewarding properties in alcohol consumption in humans and animals (52). Stress cues and negative images decrease limbic and prefrontal activity and intrinsic connectivity in abstinent AUD subjects (53, 54). Abstinence in AUD is also associated with lower striatal D2/3 (55) and increased μ-opioid receptor availability (56) along with blunted amphetamine-induced dopamine and dexamphetamine-induced opioid striatal release (57).

Notably, our effect size is from small (cross-sectional and longitudinal analyses of all participants) to medium (longitudinal analysis of the frequent drinking group). The relationship between the sample size and effect size in large datasets has been examined (58) showing that as the sample size increase, across all brain wide associations with behaviors, the effect size stabilized at around $r = 0.01$. The strongest correlation between a brain metric and a behavioral measure was $|r|=0.16$, using Adolescent Brain Cognitive Development (ABCD) Study3 (N=11,878) (58). Our effect size matches their findings. In addition, there is a critical distinction between population risk and individual risk. A statistically significant relationship might occur at the population level, but might reveal very little about the likely impact on an individual member of the population (59). In a population level study, the impact of a factor depends not only on the magnitude (effect size), but also on the distribution of the factor. Given the common use of alcohol, the small effect size may have a considerable impact at the population level (59).

Limitations and Conclusions

The Biobank data represents a normative population from 45 to 81 years with a range of alcohol use and may not be generalizable to a younger population or more severe alcohol misuse. Restricted by the available behavioral tests provided by Biobank, we used alcohol intake frequency instead of AUDIT scores in the longitudinal analysis. However, we show a strong correlation between AUDIT and the 1st timepoint alcohol intake frequency scores and, the same brain correlates are found using either measure. We have two timepoints of behavioral scores as the longitudinal measure since Biobank tested twice prospectively, which ideally could include more timepoints. For the longitudinal analysis, GRF voxel $p < 0.01$ is on the liberal side (60). However, we would like to highlight that it is based on a large sample with a medium effect size suggesting the effect is meaningful and not trivial.

Put together, we show a dissociation between dorsal and ventral mesial prefrontal, limbic substrates, and their core cognitive processes in adult alcohol misuse cross-sectionally or longitudinally. These findings have important implications for anatomical targeting for novel neuromodulatory approaches.

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Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

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Figure legends

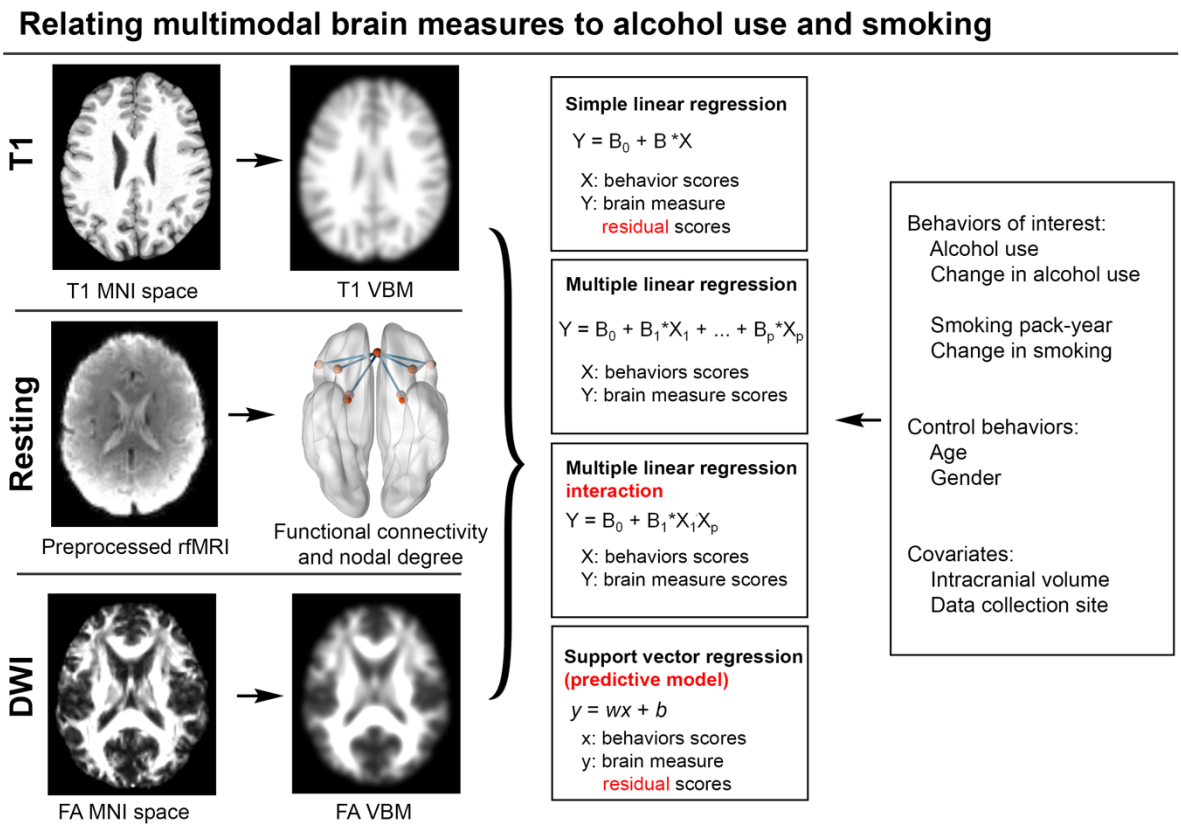

Figure 1. Analysis flowchart. Multimodal brain imaging data, including T1 grey matter concentration, resting-state fMRI functional connectivity and nodal degree, and diffusion-weighted imaging (DWI) fractional anisotropy (FA), were related to alcohol use in different regression models. Support vector regression with split-half cross-validation was used to test how well the alcohol use – brain relationship can be cross-validated and replicated. Smoking behavior was analyzed in the same pipeline as a comparison to alcohol use and to identify the common substance misuse effects on the brain. Age, gender, intracranial volume, and participants data collection site were controlled for in all models. In addition, age was analyzed in the same pipeline as alcohol use, so that we could compare the alcohol use effect with normal aging.

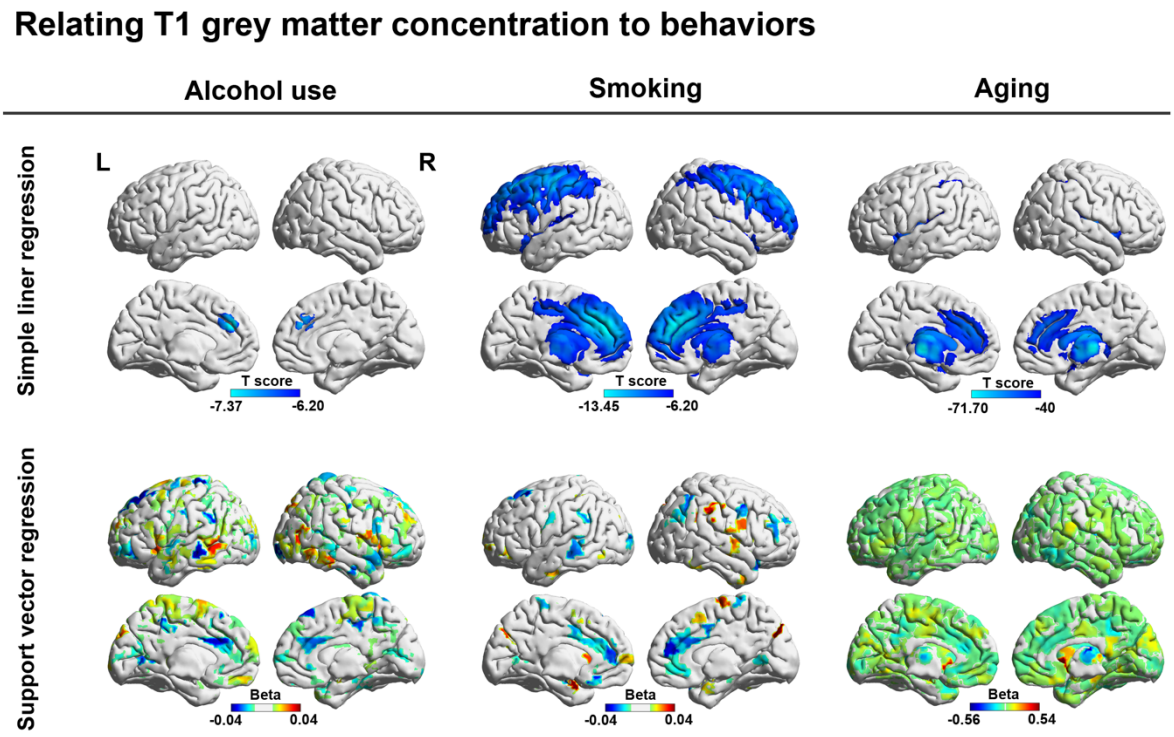

Figure 2. T1 voxel-based morphometry (VBM) cross-sectional analysis. The first row shows the brain correlates from simple linear regression and the second row shows the average brain maps (training sets) from the support vector regression with split-half cross-validation. In the first row, for alcohol use, smoking, and aging, the dorsal anterior cingulate and dorsomedial prefrontal cortex (dACC/dmPFC) are significant for three behaviors in a negative direction, which means more alcohol use, smoking, and aging related to the smaller grey matter concentration (for alcohol use and smoking, Bonferroni corrected voxel $p < 0.001$ and cluster size > 400 mm^3, for aging, a higher threshold, $T < -40$, was applied to identify the peak relationships). The alcohol effect is very localized, whilst for smoking, related regions also extend to the dorsolateral prefrontal cortex, insular, and striatum. For aging, the peak related regions are mainly surrounding the ventricles and large sulcus, including insular and striatum. The brain correlates in the simple linear regression overlap with the peak regions identified in the support vector regression.

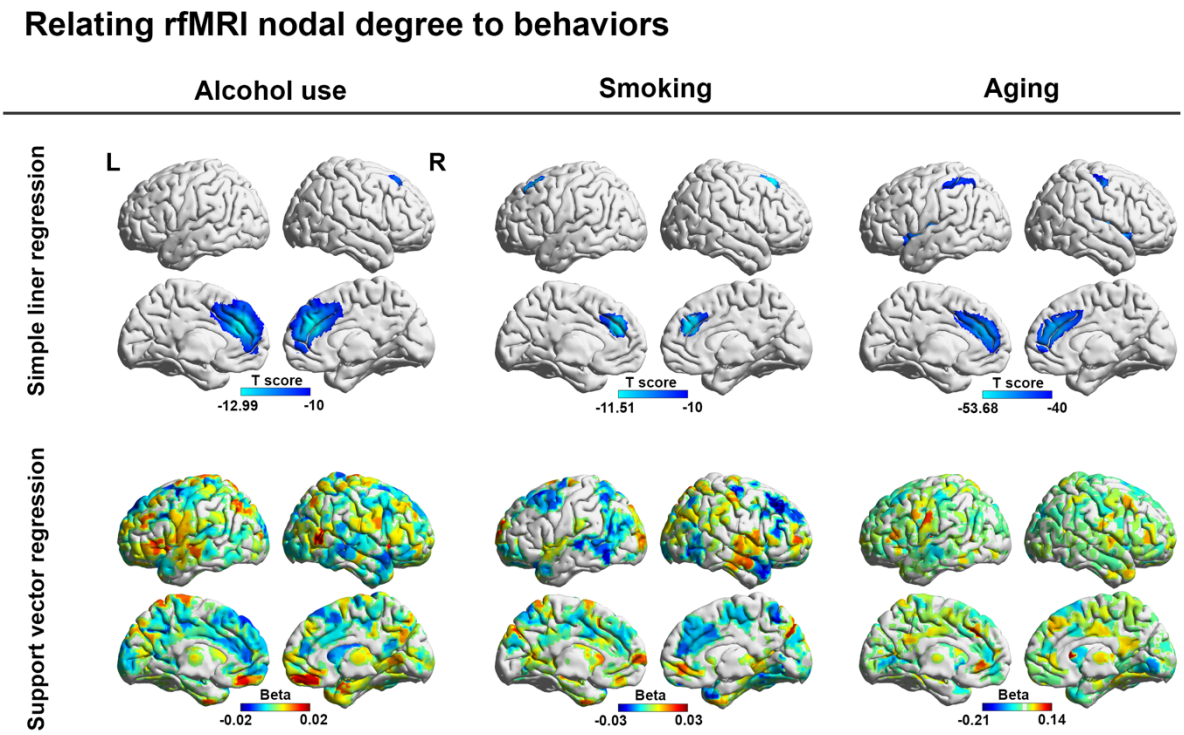

Figure 3. Relating resting-state fMRI (rfMRI) nodal degree to behaviors cross-sectional analysis. In simple linear regression, for alcohol use, smoking, and aging, the dACC/dmPFC are significant for all three behaviors in a negative direction which means more alcohol use, smoking, and aging related to the smaller nodal degree (for alcohol use and smoking, $T < -10$, $p < 10 \wedge 17$, and cluster size > 400 mm^3, for aging, a higher threshold, $T < -40$, was applied to identify the peak relationships). For smoking, bilateral superior and middle frontal gyri are also related. For aging, the peaks relationships are surrounding the ventricles and sulcus, including bilateral insular and parietal lobule. The brain correlates in the simple linear regression overlap with the peak regions identified in the support vector regression.

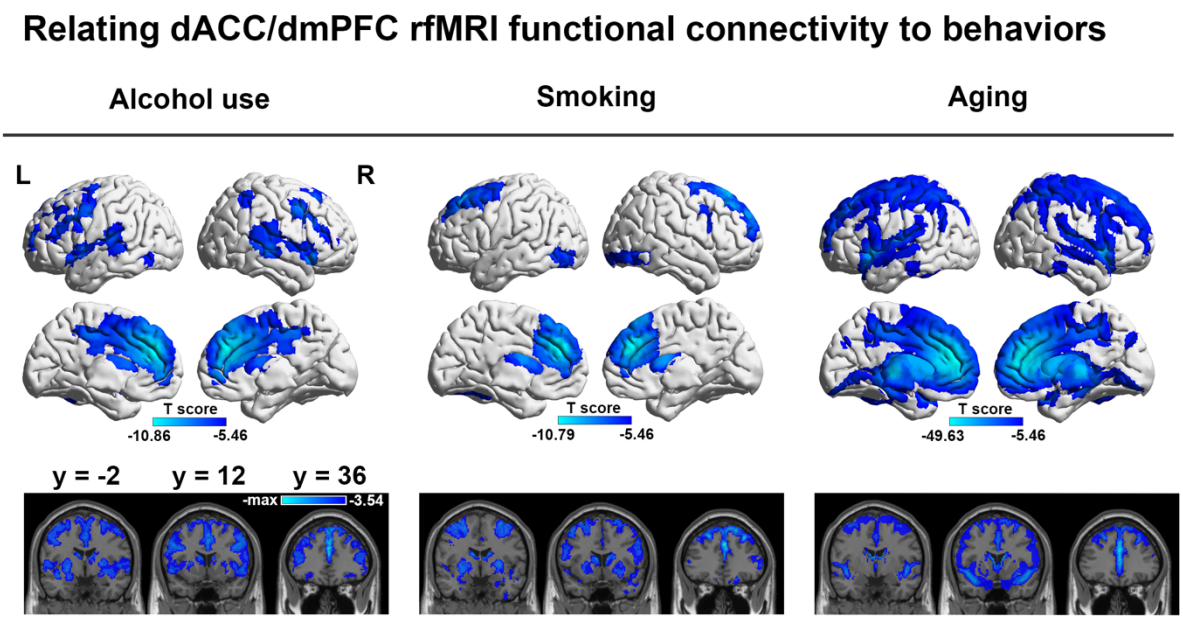

Figure 4. Relating resting-state fMRI (rfMRI) functional connectivity to behaviors cross-sectional analysis. The first row shows the brain correlates in the lateral and medial views (Bonferroni corrected voxel $p < 0.01$) and the second row shows the relationships in the subcortical regions (FDR corrected voxel $p < 0.001$). Reduced connectivity with bilateral inferior frontal, dorsolateral prefrontal and lateral orbitofrontal cortices were specific to alcohol use and unrelated to smoking or aging, whereas reduced connectivity with bilateral superior frontal gyri and medial orbitofrontal cortices was specific to smoking. In contrast, dACC/dmPFC decreased subcortical connectivity with putamen, caudate, thalamus and amygdala were observed for both alcohol and smoking but unrelated to aging highlighting common subcortical effects across substance misuse.

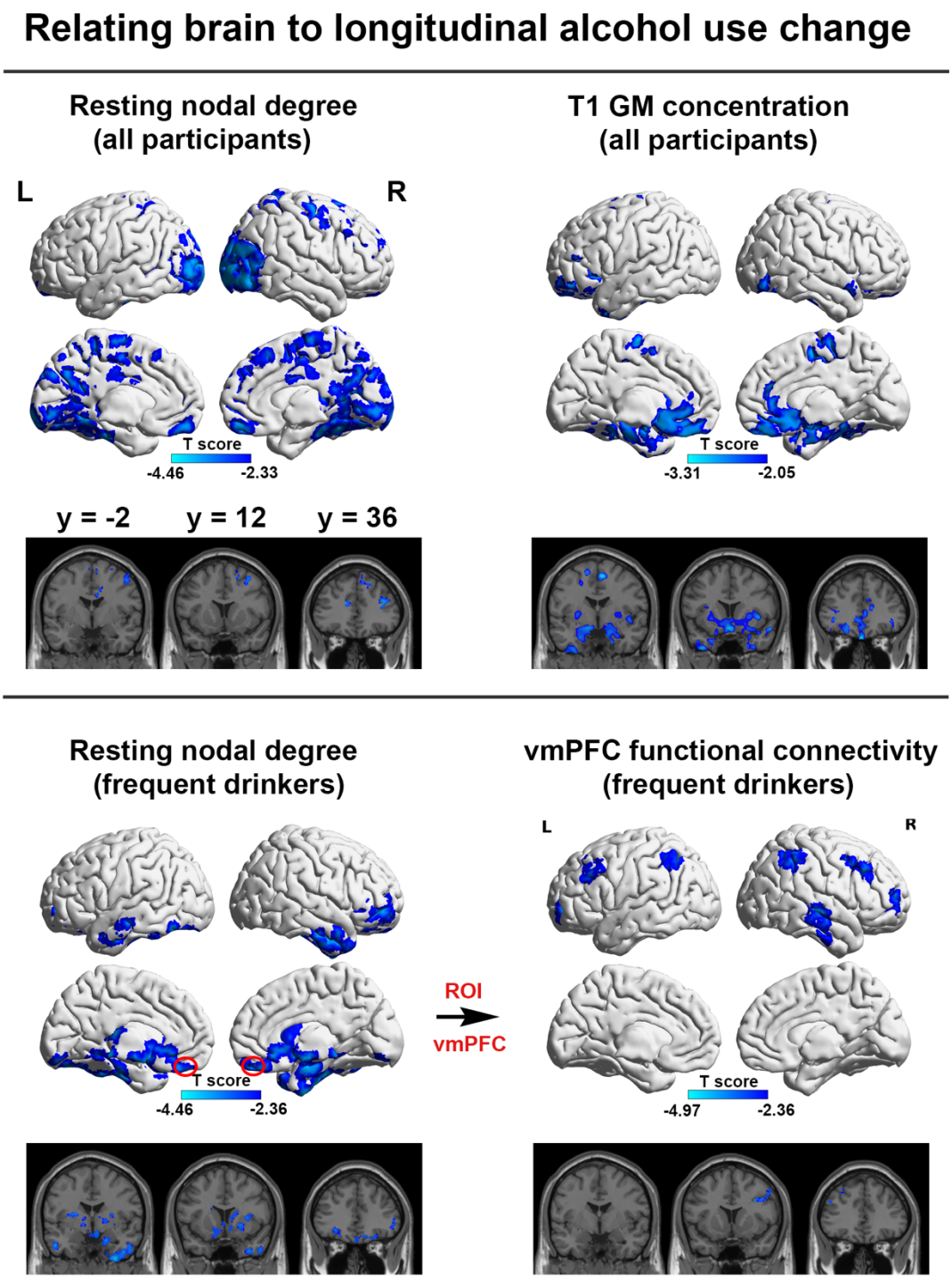

Figure 5. Relating brain to longitudinal alcohol use change. The first row two brain maps show the regions that related to alcohol use change in resting-state nodal degree (GRF corrected voxel $p < 0.01$ and cluster $p < 0.05$) and grey matter concentration (GRF corrected voxel $p < 0.02$ and cluster $p < 0.05$) analyses. The ventromedial prefrontal cortex (vmPFC) shows significance in both maps. We further separate the participants

into six groups based on their drinking frequency in the first visit (i.e. group 1 = never drinks, group 2 = special occasions only, group 3 = one to three times a month, group 4 = once or twice a week, group 5 = three or four times a week, group 6 = daily or almost daily) and then relate the brain measures to alcohol intake frequency change in each group. The result shows that only group 6, the frequent drinkers group, drives the nodal-degree relationships, as shown in the second-row first brain map (GRF corrected voxel $p < 0.01$ and cluster $p < 0.05$). The significant vmPFC cluster (in red circle) is used as a seed to calculate its whole brain functional connectivity. The vmPFC functional connections that relate to reduced drinking frequency are shown in the second-row second brain map (GRF corrected voxel $p < 0.01$ and cluster $p < 0.05$). The connected regions match to the frontal-parietal central executive network, including bilateral superior and middle frontal gyri, and inferior parietal lobule.