Multimodal brain imaging study of 19,825 participants reveals adverse effects of moderate drinking

One Sentence Summary: Moderate alcohol intake, consuming two or more units of alcohol per day, has negative effects on brain health.

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ABSTRACT

Alcohol consumption can have significant deleterious consequences, including brain atrophy, neuronal loss, poorer white matter fiber integrity, and cognitive decline, but the effects of light-to-moderate alcohol consumption on brain structure remain unclear. Here we examine the associations between alcohol intake and brain structure using structural, diffusion tensor, and neurite orientation dispersion and density imaging data from 19,825 generally healthy middle-aged and older adults from the UK Biobank. Systematically controlling for potential confounds, we found that greater alcohol consumption was associated with lower global gray and white matter volume, regional gray matter volume in cortical and subcortical areas, and white matter fiber integrity and complexity. *Post hoc* analyses revealed that these associations were non-linear. Our findings extensively characterize the associations between alcohol intake and gray and white matter macrostructure and microstructure. Consuming two or more units of alcohol per day, equivalent to one drink in some establishments, could have negative effects on brain health, an important public health finding.

Converging lines of research provide compelling evidence that chronic, excessive alcohol consumption is associated with global brain atrophy and regional brain changes.[1–3] Recent meta-analyses of magnetic resonance imaging (MRI) findings show that individuals with alcohol use disorder (AUD) have less global white matter volume (WMV)[4] and less gray matter volume (GMV) - both globally and locally in corticostriatal-limbic regions[5] - than healthy controls. Further, in a meta-analysis of pooled, multinational datasets from 33 imaging sites, individuals with AUD had lower local thickness and surface area of the hippocampus, thalamus, putamen, and amygdala than controls.[6]

In studies using diffusion-weighted MRI (dMRI), which allows a non-invasive investigation of white matter microstructure via measures of water molecule diffusion, individuals with AUD had lower fractional anisotropy (FA; the directional coherence of water molecule diffusion) and greater mean diffusivity (MD; the magnitude of water molecule diffusion) in the corpus callosum, frontal forceps, internal and external capsules, fornix, superior cingulate, and longitudinal fasciculi than controls.[1,7] However, because conventional dMRI measures (FA and MD) are based on a simplistic model of brain tissue microstructure, they fail to account for the complexities of neurite geometry.[8] For example, the lower FA observed in individuals with AUD[1,7] may reflect lower neurite density and/or greater orientation dispersion of neurites, which conventional dMRI measures do not differentiate.[9,10] A key question raised by prior findings in individuals with AUD that remains is whether, similar to heavy drinking, light-to-moderate alcohol consumption adversely affects brain structure. Further, is the relationship between alcohol intake and brain structure linear? In some studies of middle-aged and older adults, moderate alcohol consumption was associated with lower total cerebral volume,[11] gray matter atrophy,[12,13] and lower density of gray matter in frontal and parietal brain regions.[13] However, other studies have shown no association,[14] and one study showed a *positive* association between light-to-moderate alcohol consumption and GMV in older men.[15] One interpretation of these findings is that a U-shaped, dose-dependent association exists between alcohol use and

brain morphometry, with light-to-moderate drinking being protective against and heavy drinking being a risk factor for lower GMV.[15,16] However, these results are inconclusive, as a longitudinal cohort study[17] showed no difference in structural brain measures between abstinent individuals and light drinkers, while moderate-to-heavy drinkers showed GMV atrophy in the hippocampi and impaired white matter microstructure (lower FA, higher MD) in the corpus callosum.

The inconclusive nature of the evidence regarding the association between moderate alcohol intake and brain structure may reflect the patchwork nature of the literature, which consists of mostly small, unrepresentative studies with limited statistical power.[18,19] Moreover, most studies to date have not accounted for the effects of many relevant covariates and therefore have yielded findings with limited generalizability. Potential confounds that may be associated with individual differences in both alcohol intake and neuroanatomy include sex,[20] body mass index (BMI),[21] age,[22] and genetic population structure (i.e., biological characteristics that are correlated with environmental causes).[23] Similar to other fields of research, progress in this area may also be limited by publication bias.[24]

The current study used data from nearly 20,000 participants in the UK Biobank (UKB) to characterize the associations between alcohol intake (i.e., mean units per day; one unit=10 ml of pure ethanol) and brain structure (total GMV and WMV, regional GMV) and white matter microstructure in the 27 major tracts (Fig. 1). We addressed the limitations of the existing literature through a pre-registered analysis of multimodal imaging data from the UKB.[25–27] The UKB, a prospective cohort study representative of the United Kingdom (UK) population aged 40-69 years, is the largest available collection of high-quality MRI brain scans, alcohol-related behavioral phenotypes, and measurements of the socio-economic environment. Participants self-reported their usual weekly alcohol consumption through a touch screen questionnaire,[26] from which we calculated mean alcohol intake. A subsample of participants completed a brain imaging scan session that included three structural modalities, resting and task-based fMRI, and diffusion-weighted imaging.[25–27] Importantly, the dMRI measures available in the UKB include

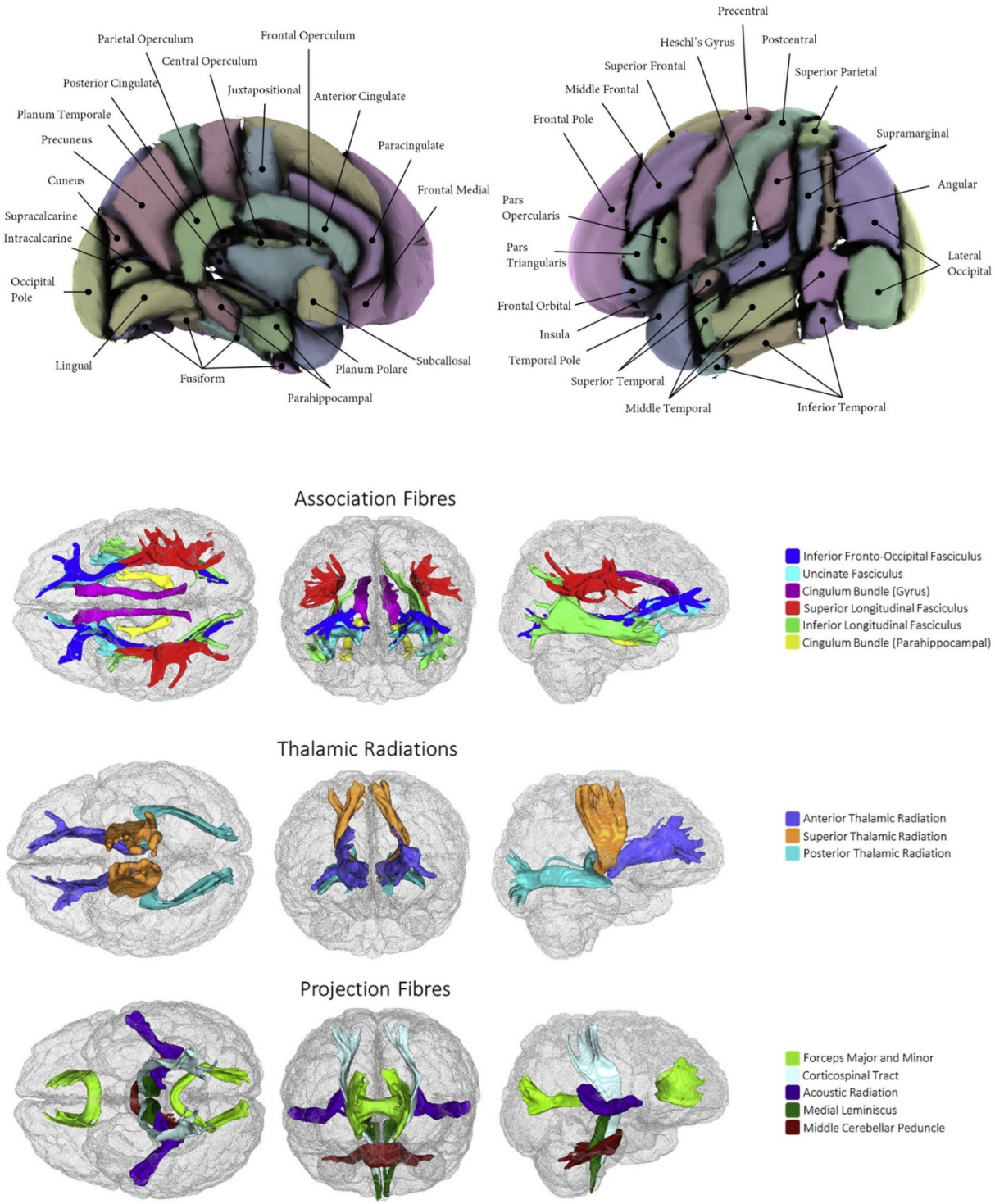

Figure 1. Brain imaging regions of interest according to the Harvard-Oxford Atlas (top: cortical regions) and AutoPtx (bottom: white matter tracts) from Cox and colleagues (2019).[40]

the conventional metrics of FA and MD, but also neurite orientation dispersion and density imaging (NODDI).[10] Such measures offer information on white matter microstructure and

estimates of neurite density (i.e., intra-cellular volume fraction; ICVF), extracellular water diffusion (i.e., isotropic volume fraction; ISOVF), and tract complexity/fanning (i.e., orientation dispersion, OD). This allowed us to assess the nature of alcohol's effects on white matter microstructure in greater detail than any previous studies on the topic.

The richness and scale of the UKB dataset also enabled us to control for many important confounds, including genetic population structure, and to estimate small effects accurately, including those of moderate drinking on brain structure. Because regular moderate drinking is the most common pattern of consumption in the UK, where 57% of adults, or an estimated 29.2 million individuals, reported drinking in the previous week,[28] our findings have important public health implications for the UK and other countries where alcohol is commonly consumed.

RESULTS

Characteristics of the 19,825 participants (52.5% female) are shown in Table 1. Participants were healthy middle-aged and older adults.

We first estimated a series of linear regressions with alcohol intake as the main explanatory variable of interest and imaging-derived phenotypes (IDPs) extracted by the UKB brain imaging processing pipeline[29] as the dependent variables, controlling for sex, age, age-squared, age-cubed, height, total brain volume (grey+white matter, for volumetric data only), the Townsend index of social deprivation measured at the zip code level[30] and handedness. Family-wise error (FWE) correction of the *p*-values was applied using the Holm method.[31] This analysis was pre-registered in the Open Science Network (https://osf.io/trauf/?view_only=a3795f76c5a54830b2ca443e3e07c0f0).

We measured alcohol intake in *log(1 + units/day)* with one unit representing 10 ml of pure ethanol and found that it was associated with lower global GMV (standardized β=-0.080 [95% CI -0.093 to -0.067], t=-12.17, $p<1.0\times10^{-6}$) and lower global WMV (standardized β=-0.044 [95% CI -0.059 to -0.028], t=-5.70, $p<1.0\times10^{-6}$). When controlling for total brain volume, we also identified negative associations between alcohol intake and regional GMV in 16 brain regions

Table 1. Descriptive characteristics of the population

	Study Sample *N* = 19,825	Heavy Drinkers *n* = 1,226	Abstainers *n* = 1,527	Test Statistic
Mean age (y) (SD)	62.7 (7.4)	62.7 (7.0)	63.3 (7.5)	t=-2.14*
Sex [n, (%) women]	10,406 (52.5)	488 (39.8)	1,058 (69.2)	z=15.47**
Population group (% white)	100	100	100	··
Education (y) (SD)	13.5 (4.0)	13.7 (4.1)	13.4 (4.1)	t=1.87
Alcohol units per week (SD)	8.2 (8.2)	29.7 (9.4)	0.0	··
BMI (SD)	26.6 (4.3)	27.3 (3.9)	27.2 (5.4)	t=0.76
Total GMV + WMV (cm^3) (SD)	1,167.6 (111.3)	1,169.8 (104.8)	1,140.4 (109.9)	t=7.19**
Total GMV (cm^3) (SD)	616.7 (55.3)	615.6 (52.3)	604.4 (55.2)	t=4.74**
Total WMV (cm^3) (SD)	551.6 (62.0)	556.6 (59.1)	536.1 (60.1)	t=7.43**

Note: **p*-value<0.05, ** *p*-value<0.001, z-statistic (proportions) and *t*-statistic (means) between heavy drinkers and abstainers. BMI: body mass index, GMV: gray matter volume, WMV: white matter volume, y: years

(standardized β range -0.048 to -0.020) (Supplementary Table 1) demonstrating local associations that were above and beyond the global effects. Alcohol intake was also associated with poorer white matter microstructure (lower FA, ICVF, and OD; higher MD and ISOVF) (Supplementary Tables 2 and 3). Additional analyses that adjusted also for weight, BMI, and educational attainment, revealed one additional association between alcohol intake and regional GMV (left precentral gyrus, standardized β = -0.027 [95% CI -0.039 to -0.016], t = -4.076, p = 2.55 x 10^{-6}) and an association between alcohol intake and ICVF in the bilateral anterior thalamic radiation (left: standardized β = -0.027 [95% CI -0.041 to -0.013], t = -3.711, p = 2.07 x 10^{-4}; right: standardized β = -0.028 [95% CI -0.043 to -0.014, t = -3.888, p = 1.01 x 10^{-4}), whereas the association between alcohol intake and the left lateral occipital cortex was no longer statistically significant. The remaining regional associations with alcohol intake were, in general, smaller than the global effects.

The strongest regional GMV effects identified above the global effects were in the bilateral putamen (left: standardized β = -0.051 [95% CI -0.065 to -0.037], t = -7.087, p = 1.42 x

10^{-12}; right: standardized β = -0.047 [95% CI -0.061 to -0.033], *t* = -6.664, *p* = 2.72 x 10^{-11}) and brain stem (standardized β = -0.033 [95% CI -0.045 to -0.020], *t* = -5.299, *p* = 1.18 x 10^{-7}). Some of our linear regressions showed *positive* associations between drinking and regional GMV relative to the global effect. Specifically, greater alcohol intake was associated with greater regional GMV in the bilateral pallidum (left: standardized β = 0.029 [95% CI 0.015 to 0.043], *t* = 3.938, *p* = 8.23 x 10^{-5} right: standardized β = 0.033 [95% CI 0.018 to 0.047], *t* = 4.473, *p* = 7.76 x 10^{-6}), right inferior temporal gyrus (standardized β = 0.026 [95% CI 0.013 to 0.038], *t* = 4.040, *p* = 5.35 x 10^{-5}), and left lingual gyrus (standardized β = 0.023 [95% CI 0.013 to 0.034], *t* = 4.242, *p* = 2.23 x 10^{-5}). We estimated additional regression models to determine whether these associations were positive in absolute value, or only relatively to the global effects. After removing total brain volume as a control variable from the linear regression models, the associations between alcohol intake and these regional GMV IDPs were no longer significant. This finding suggests that the association between alcohol intake and brain structure is negative, and likely occurs in stages over time, with alcohol intake affecting specific, perhaps more vulnerable, brain regions before influencing other regions (e.g., bilateral pallidum).

In our analyses using dMRI IDPs, alcohol intake was associated with lower FA and higher MD in the bilateral posterior thalamic radiation fibers and forceps minor (Supplementary Table 2). Associations with thalamic radiation fibers (anterior, posterior, and superior) and the forceps minor (Fmin) were among the largest in magnitude and found across all white matter measures. As shown in Supplementary Tables 2 and 3, there were also associations between alcohol intake and MD, ISOVF, ICVF, and/or OD in several association fibers [inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF); superior longitudinal fasciculus (SLF); uncinate], and projection fibers [acoustic radiation (AR); forceps major (Fmaj); corticospinal tract (CST); middle cerebellar peduncle (MCP)].

Pre-registered sensitivity analyses that re-estimated our regression models while excluding heavy drinkers or non-drinkers altered the sign and/or magnitude of several of the

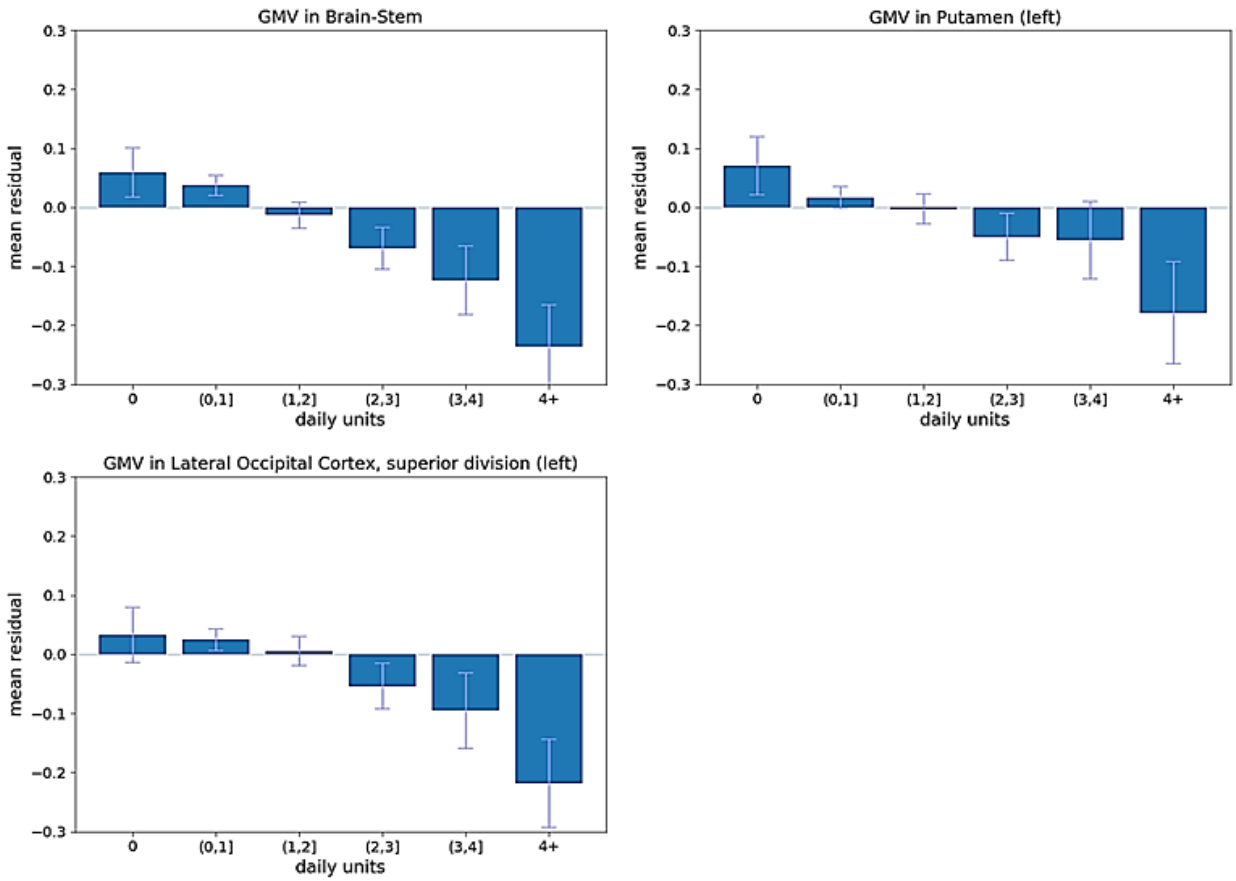

FIGURE 2. Average regional gray matter volume based on daily drinking levels in the brain stem, left putamen, and left lateral occipital cortex**.** Daily unit = 10 ml of pure ethanol.

effects that we observed, suggesting that the associations vary non-linearly across the drinking range. Thus, after we regressed out the effects of covariates (this time excluding brain volume, in order to determine absolute effects), we grouped participants into five bins based on their average daily drinking level (0, 0-1, 1-2, 2-3, 4+ units) and quantified the average levels of the IDPs in each group (Fig. 2 and Supplementary Figs 1-5). The associations were mainly driven by individuals who reported consuming at least two or more units of alcohol per day, with no substantial effects of alcohol intake among individuals who reported consuming less than two units/day. Figure 2 displays association patterns in the brain stem, left putamen, and left lateral occipital cortex where individuals who consumed two or more units/day showed lower average regional GMV. The most substantial association between alcohol intake and regional GMV occurred among individuals who consumed four or more units/day. These individuals showed

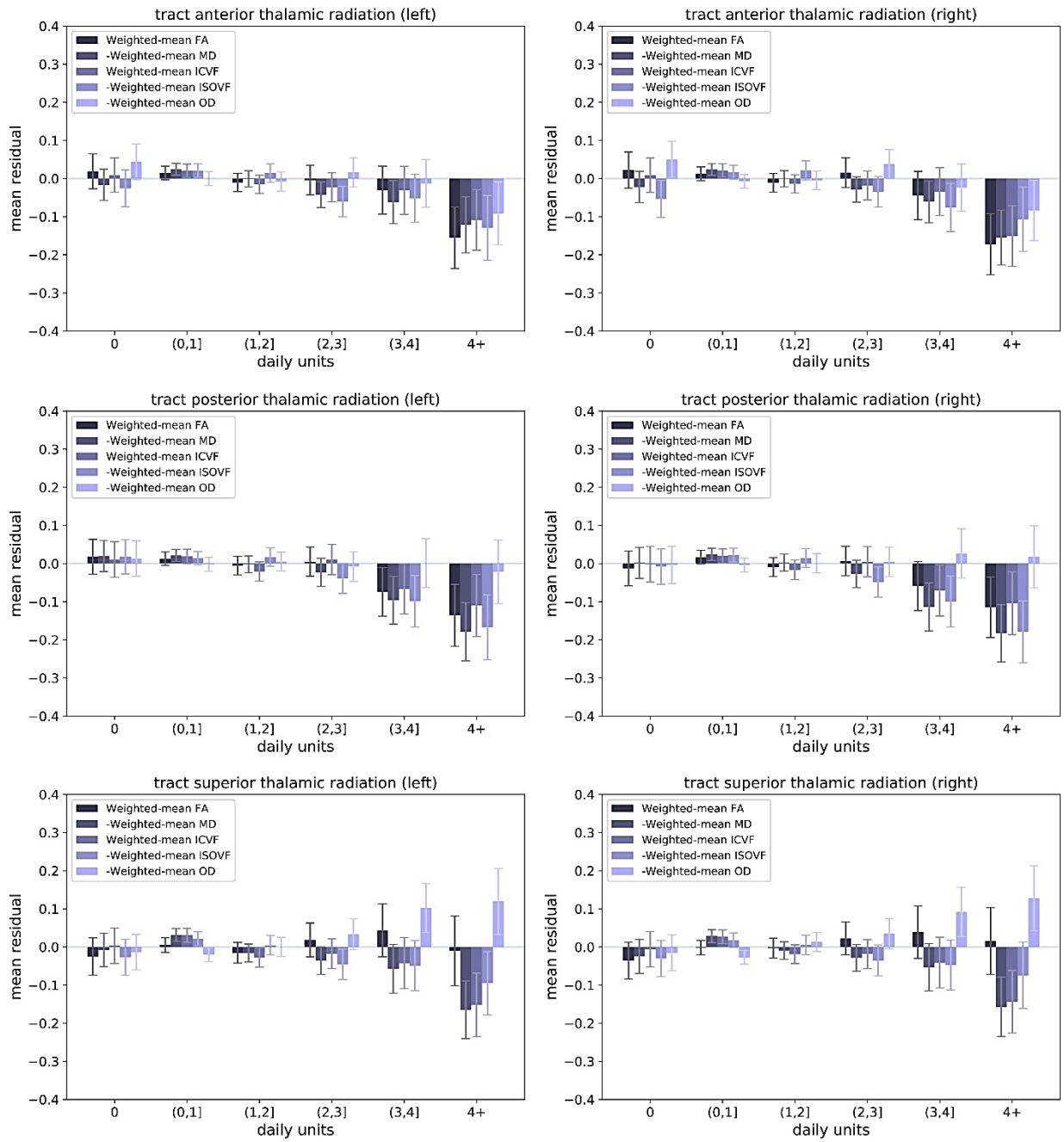

FIGURE 3. Average white matter water microstructure indices based on daily drinking levels in the bilateral thalamic radiation fibers. Daily unit = 10 ml of pure ethanol. FA = fractional anisotropy; MD = mean diffusion; ICVF = intracellular volume fraction; ISOVF = isotropic volume fraction; OD = orientation diffusion; MD, ISOVF, and OD values are represented as negative values.

lower average local GMV throughout the brain. The bilateral pallidum was the only regional GMV IDP that did not show significant differences across average daily drinking level.

We conducted similar analyses focusing on the five white matter microstructure measures across 27 white matter tracts. In Figure 3 and Supplementary Figures 6 and 7, we present the results for white matter tracts across the average daily drinking levels where the mean residuals for three or more microstructure measures were significant. The majority of associations between alcohol intake and white matter microstructure measures reflected less healthy white matter -- that is a combination of lower FA, higher MD, lower ICVF, higher ISOVF, and/or lower OD -- among individuals who reported consuming three or more units/day of alcohol. These findings were evident across bilateral thalamic radiation fibers (ATR, PTR, STR) (Fig. 3), association fibers (bilateral IFOF, bilateral ILF, bilateral SLF, right CingG, and right UNC), and projection fibers (Fmin and MCP) (Supplementary Figs. 6 and 7). A positive association between alcohol intake and FA was observed in the bilateral CST, where individuals with greater alcohol intake had higher FA than non-drinkers who consumed less than two units/day.

DISCUSSION

We conducted a multimodal brain imaging study of nearly 20,000 middle-aged and older adults of European descent, a population sample that reported alcohol consumption across the entire spectrum from abstinence to heavy drinking. The scale and granularity of the data provided ample statistical power to identify small effects and explore non-linear dependencies while accounting for important potential confounds. Associations between greater alcohol intake and poorer brain health were small but significant across global brain measures and cortical and subcortical gray matter and white matter microstructure. The comprehensiveness and sensitivity of these findings add to our understanding of the associations between alcohol intake and brain health in humans.

Although the link between alcohol intake and less healthy brain tissue was predominantly driven by heavy drinkers, effects were also observed among individuals who reported consuming two units/day of alcohol. This has important implications for

recommendations regarding safe drinking levels. In 2016, the UK Chief Medical Officers published new “low-risk” alcohol consumption guidelines that advise limiting alcohol intake to 14 units per week[32]. One unit of alcohol is equivalent to 10 ml or 8 g of ethanol, which is contained in 25 ml of 40% spirits, 250 ml of 4% beer, and 76 ml of 13% wine. Many drinking establishments serve drinks that contain 35-50 ml of 40% spirits (1.4-2 units), 568 ml of 4% beer (2.27 units), and 175 ml of 13% wine (2.30 units).[33] Thus, in the UK, consuming just one alcoholic drink (i.e., two units of alcohol) daily could have negative effects on brain health. This has important public health implications insofar as 57% of UK adults, or an estimated 29.2 million individuals,[28] endorsed past-week drinking.

Associations between measures of brain structure and alcohol intake were generally in the expected direction, providing additional evidence of the negative effects of low-to-moderate alcohol consumption on brain structure. Alcohol is a neurotoxic agent that induces brain oxidative stress,[34] alters neuroimmune response,[35] damages myelin,[7] and alters neurotransmission and neurotransmitter systems.[2] These alterations interfere with neural function, resulting in cognitive impairments, and are likely associated with changes in dendritic spine formation.[36,37] Thus, it is not surprising that low-to-moderate alcohol consumption was associated with less global GMV, global WMV, and regional GMV, and less healthy white matter structure. Although the exact mechanisms of alcohol’s neurotoxic effects are still under investigation, our findings provide the first evidence of an association between alcohol intake and neurite orientation diffusion and density. Specifically, alcohol consumption was associated with lower neurite density, lower tract complexity and greater water diffusion in thalamic radiations and association fasciculi, and may reflect the effect of alcohol on myelin and axonal fibers. Future investigations into the mechanisms underlying the neurotoxic effects of alcohol on the brain, particularly among occasional binge drinkers (e.g. college students), are warranted.

Our findings also have implications for the design and analysis of future studies using brain images in general population samples such as the UKB. A failure to account for the

effects of drinking, either by controlling for alcohol intake or excluding participants who drink more than one drink (two units of alcohol) per day (which comprised 44% of our study population), could introduce an unwelcome source of variance into the analysis. Furthermore, while neuroimaging studies commonly examine linear relationships between brain features and other explanatory variables, our results demonstrate that the linearity assumption underlying most studies could be overly simple.

Our study is not without limitations and these provide opportunities for further research. First, we relied on a sample of middle-aged individuals of European ancestry living in the UK. We hope that future work will test the generality of our findings among individuals from other populations, and in other age groups. It is reasonable to expect that the relationship we observed would differ in younger individuals who have not experienced the chronic effects of alcohol on the brain. An additional limitation stems from the self-reported measures of alcohol intake in the UK Biobank, which covers only the past year. Such estimates do not adequately reflect drinking prior to the past year and are susceptible to reporting and recall bias.[38,39]

In summary, in this comprehensive examination of the associations between alcohol intake and brain macro- and micro-structure, we uncovered multiple associations. The associations were most pronounced in heavy drinkers, yet some effects were observed among individuals who reported consuming two or more units/day of alcohol. These findings provide an extensive characterization of the associations between alcohol intake and gray and white matter macrostructure and microstructure, and offer insights into the potential effects of light-to-moderate alcohol consumption on brain architecture.

Methods

Sample

All UK Biobank (www.ukbiobank.ac.uk) participants provided written informed consent and ethical approval was granted by the North West Multi-Centre Ethics committee. Our sample

comprised 19,825 individuals of European ancestry from the UKB database whose data were available as of October 18, 2018. The number of participants included in each model decreased when phenotype data were missing. All of the structural T1 MRI images that we used passed the automated quality control of the UKB brain imaging processing pipeline.[29] We ran additional quality checks using the Computational Anatomy Toolbox (CAT; www.neuro.uni-jena.de/cat/) for SPM (www.fil.ion.ucl.ac.uk/spm/software/spm12/), which resulted in 747 individuals who exhibited substantial image inhomogeneity (i.e., overall volume correlation below two standard deviations from the mean) being removed from the analysis.

Measures of alcohol consumption

Participants self-reported the number of units of alcohol (10 ml of pure ethanol) consumed "in an average week" in several beverage categories in "units per week" (for frequent drinkers) or "units per month" (for less frequent drinkers). The UKB assessment defined units of alcohol as follows: a pint or can of beer/lager/cider = two units; a 25-ml single shot of spirits = one unit; and a standard glass of wine (175 ml) = two units. The categories are *red wine*, *white wine/champagne*, *beer/cider*, *spirits*, *fortified wine*, and "*other*". Number of weekly units was computed by summing the weekly number of units for all categories. When reported monthly, the intake was converted to units per week by dividing by 4.3. Number of weekly units was divided by seven to determine units per day.

MRI data acquisition

Participants were scanned using a Siemens Skyra 3T scanner (Siemens Healthcare, Erlangen, Germany) using a standard 32-channel head coil, according to a freely available protocol (http://www.fmrib.ox.ac.uk/ukbiobank/protocol/V4_23092014.pdf), documentation (http://biobank.ctsu.ox.ac.uk/crystal/docs/brain_mri.pdf), and publication.[40] As part of the scanning protocol, high-resolution T1-weighted images, three-dimensional T2-weighted fluid-attenuated inversion recovery (FLAIR) images, and diffusion data were obtained. High resolution T1-weighted images were obtained using an MPRAGE sequence with the following

parameters: TR=2000ms; TE=2.01ms; 208 sagittal slices; flip angle, 8°; FOV=256 mm; matrix=256×256; slice thickness=1.0mm (voxel size 1×1×1mm); total scan time=4min 54s. 3D FLAIR images were obtained with the following parameters: TR=1800ms; TE=395.0ms; 192 sagittal slices; FOV=256mm; 256×256; slice thickness=1.05mm (voxel size 1.05×1×1mm); total scan time=5min 52s. Diffusion acquisition comprised a spin-echo echo-planar sequence with 10 T2-weighted ($b \approx 0$ s mm^{-2}) baseline volumes, 50 $b = 1000$ s mm^{-2} and 50 $b = 2000$ s mm^{-2} diffusion weighted volumes, with 100 distinct diffusion-encoding directions and 2 mm isotropic voxels; total scan time=6min 32s.

MRI data preprocessing

Structural imaging and diffusion data were processed by the UK Biobank team and made available to approved researchers as imaging-derived phenotypes (IDPs); the full details of the image processing and QC pipeline are available in an open access article.[25,29] IDPs used in analyses included total brain volume, gray matter volume, white matter volume, 139 regional GMV IDPs derived using parcellations from the Harvard-Oxford cortical and subcortical atlases and Diedrichsen cerebellar atlas (UKB fields 25782 to 25920), and tract-averaged measures of fractional anisotropy (FA), mean diffusivity (MD), intra-cellular volume fraction (ICVF), isotropic volume fraction (ISOVF), and orientation diffusion (OD). White matter measures were used from the following white matter tracts: middle cerebellar peduncle (MCP), forceps major (FMaj), forceps minor (FMin) and bilateral medial lemnisci, corticospinal tract (CST), acoustic radiation (AR), anterior thalamic radiation (ATR), posterior thalamic radiation (PTR), superior thalamic radiation (STR), superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF) and inferior fronto-occipital fasciculus (IFOF), and both the cingulate gyrus and parahippocampal portions of the cingulum bundle. Individuals whose IDPs were more than four standard deviations from the mean were excluded from analyses.

Statistical analyses

We pre-registered the analysis plan (https://osf.io/trauf/?view_only=a3795f76c5a54830b2ca443e3e07c0f0). Our main analysis (model A) estimated a linear regression with the IDPs as dependent variables and alcohol intake (log(1 + units/week)) as the main independent variable of interest, controlling for sex, age, age-squared, age-cubed, height, brain volume, the Townsend index of social deprivation measured at the zip code level[30] and handedness (right/left/ambidextrous; dummy-coded). To control for genetic population structure, the models also included the first 40 genetic principal components,[41] and dummy-coded county of residence.[42] All continuous variables (except age-related variables) were standardized to a mean of 0 and a standard deviation of 1. We also performed three sensitivity analyses. Model (B) included additional control for variables that are potential downstream effects associated with alcohol intake: weight, body mass index (BMI), and educational attainment.[43] The two other models repeated the analysis of model (A), with Model (C) excluding non-drinkers and model (D) excluding heavy drinkers (i.e., women who reported consuming more than 18 units/week and men consuming more than 24 units/week). Once we identified IDPs that were robustly associated with alcohol intake using linear regression models, we investigated whether the associations were dose dependent. For example, deleterious effects of alcohol on GMV of a specific brain region could occur only in heavy drinkers. Hence, we binned participants in the following six categories based on average alcohol intake: (1) abstainers (*n*=1,527), (2) individuals who drank less than one unit/day (*n*=9,595) (3) individuals who drank between one (included) and two (excluded) units/day (maximal amount recommended, *n*=5,189) (4) individuals who drank between two (included) and three (excluded) units/day (*n*=2,215), (5) individuals who drink between three (included) and four (excluded) units/day (*n*=805), and (6) individuals who drink at least four units/day (*n*=568). We then calculated the mean IDP values (after regressing the influence of all control variables specified in model A) and 95% confidence intervals (CI) around them.

Statistical significance

To control the family-wise error rate, we determined the significance thresholds for all regressions using the Holm method[31] and included the results from all IDPs.

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Author contributions

RD, PK, HRK, GN, and RW conceived of and designed the study. RD analyzed data. RD, GA, KJ, PK, HRK, GN, and RRW interpreted data. RD and RRW wrote the paper. GA, NS, PK, HRK, and GN critically edited the work. RRW edited the work. All authors approved the final version to be submitted for publication and agree to be accountable for all aspects of this work.

Competing interests

HRK is a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which was supported in the last three years by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, Pfizer, Arbor, and Amygdala Neurosciences and is named as an inventor on PCT patent application #15/878,640 entitled: "Genotype-guided dosing of opioid agonists," filed January 24, 2018. All other authors declare no competing interests.

Supplementary Materials for

Multimodal brain imaging study of 19,825 participants reveals adverse effects of moderate drinking

Supplementary Table 1. Associations between alcohol intake and regional gray matter volume imaging-derived phenotypes

		Model A			Model B			Model C			Model D		
	L/R	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***
Frontal pole	L	-0.020	-4.552	5.34E-05	-0.020	-4.464	8.09E-06	-0.021	-3.941	8.15E-05	-0.020	-4.017	5.91E-05
Precentral gyrus	L	-0.027	-4.636	3.76E-06	-0.027	-4.706	2.55E-06	-0.033	-4.779	1.77E-06	··	··	··
Precentral gyrus	R	-0.031	-5.355	8.66E-08	-0.032	-5.508	3.67E-08	-0.042	-6.162	7.34E-10	-0.026	-3.975	7.06E-05
Temporal pole	R	-0.024	-4.081	4.50E-05	-0.024	-4.015	5.97E-05	-0.030	-4.310	1.64E-05	··	··	··
Inferior temporal gyrus	R	0.025	3.893	9.93E-05	0.026	4.040	5.35E-05	··	··	··	··	··	··
Superior temporal gyrus	L	··	··	··	··	··	··	-0.032	-4.310	1.64E-05	··	··	··
Postcentral gyrus	L	··	··	··	-0.023	-3.755	1.74E-04	-0.029	-3.995	6.50E-05	··	··	··
Postcentral gyrus	R	-0.024	-3.933	8.41E-05	-0.025	-3.968	7.27E-05	-0.035	-4.802	1.58E-06	··	··	··
Lateral occipital cortex	L	-0.021	-3.685	2.29E-04	··	··	··	··	··	··	··	··	··
Lateral occipital cortex	R	-0.247	-4.282	1.86E-05	-0.024	-4.106	4.04E-05	··	··	··	-0.022	-3.394	6.90E-04
Cuneal cortex	L	-0.029	-4.369	1.25E-05	-0.027	-4.167	3.10E-05	-0.032	-4.110	3.97E-05	-0.028	-3.938	8.25E-05
Frontal orbital cortex	R	-0.021	-3.740	1.84E-04	-0.021	-3.702	2.15E-04	-0.027	-4.108	4.02E-05	··	··	··
Lingual gyrus	L	0.025	4.446	8.81E-06	0.023	4.242	2.23E-05	··	··	··	0.022	3.581	3.43E-04
Central opercular cortex	R	··	··	··	··	··	··	-0.026	-3.905	9.45E-05	··	··	··
Planum polare	L	-0.024	-4.048	5.18E-05	-0.026	-4.259	2.06E-05	-0.028	-3.895	9.87E-05	··	··	··
Planum polare	R	-0.027	-4.701	2.61E-06	-0.028	-4.929	8.34E-07	-0.032	-4.703	2.58E-06	-0.023	-3.633	2.80E-04
Heschl's gyrus	R	-0.024	-4.048	5.18E-05	-0.026	-4.387	1.16E-05	-0.026	-3.715	2.03E-04	··	··	··
Putamen	L	-0.048	-6.711	1.99E-11	-0.051	-7.087	1.42E-12	-0.050	-5.946	2.79E-09	-0.041	-5.246	1.57E-07
Putamen	R	-0.043	-6.079	1.23E-09	-0.047	-6.665	2.72E-11	-0.047	-5.646	1.67E-08	-0.031	-3.909	9.29E-05
Pallidum	L	0.030	4.105	4.06E-05	0.029	3.938	8.23E-05	··	··	··	0.029	3.930	8.52E-05
Pallidum	R	0.034	4.726	2.31E-06	0.033	4.473	7.76E-06	0.036	4.144	3.43E-05	0.036	4.741	2.14E-06
Amygdala	R	-0.024	-4.322	1.55E-05	-0.027	-4.878	1.08E-06	-0.032	-4.772	1.84E-06	··	··	··
Brain stem		-0.033	-5.368	8.06E-08	-0.033	-5.299	1.18E-07	-0.034	-4.619	3.88E-06	-0.032	-4.949	7.53E-07
V Cerebellum	L	··	··	··	··	··	··	··	··	··	0.026	3.630	2.84E-04

Note. Reported β values are standardized. GMV: gray matter volume, IDP: imaging-derived phenotype, L/R: left/right, *t*: t-value; *p*: p-value. Model A (*n*=19,825) estimated a linear regression with the IDPs as dependent variables and alcohol intake as the main independent variable of interest, controlling for sex, age, age-squared, age-cubed, height, brain volume, the Townsend index of social deprivation measured at the zip code level, handedness (right/ left/ ambidextrous; dummy-coded), the first 40 genetic principal components,[1] and dummy-coded county of residence ($p < 2.43 \times 10^{-4}$).[2] All continuous variables (except age-related variables) were standardized to a mean of 0 and a standard deviation of 1. Model B (*n*=19,825) included additional control for variables that are potentially associated with alcohol intake: weight, body mass index, and educational attainment ($p < 2.34 \times 10^{-4}$). Model C (*n*=18,298) repeated model A, excluding non-drinkers ($p < 2.13 \times 10^{-4}$). Model D (*n*=18,599) repeated model A, excluding heavy drinkers (i.e., women who reported consuming more than 18 units/week and men who reported consuming more than 24 units/week[3] ($p < 7.25 \times 10^{-4}$).

Supplementary Table 2. Associations between alcohol intake and white matter water molecular diffusion indices (FA and MD)

		Model A			Model B			Model C			Model D		
Tract	**L/R**	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***
Fractional Anisotropy (FA)													
CingG	R	-0.033	-4.354	1.35E-05	-0.034	-4.479	7.54E-06	-0.037	-4.067	4.78E-05	··	··	··
PTR	L	-0.035	-4.584	4.58E-06	-0.037	-4.948	7.58E-07	-0.040	-4.509	6.55E-06	··	··	··
PTR	R	-0.032	-4.182	2.91E-05	-0.033	-4.396	1.74E-05	-0.045	-4.899	9.71E-07	··	··	··
Fmin		-0.031	-4.129	3.66E-05	-0.032	-4.272	1.95E-05	-0.034	-3.846	1.20E-04	··	··	··
Mean Diffusivity (MD)													
ILF	R	··	··	··	··	··	··	0.040	4.574	4.82E-06	··	··	··
SLF	L	0.028	3.783	1.56E-04	0.028	3.834	1.26E-04	0.039	4.440	9.06E-06	··	··	··
SLF	R	0.029	3.885	1.03E-04	0.029	3.901	9.61E-05	0.043	4.900	9.68E-07	··	··	··
ATR	L	··	··	··	··	··	··	0.032	3.855	1.16E-04	··	··	··
ATR	R	··	··	··	··	··	··	0.032	3.866	1.11E-04	··	··	··
PTR	L	0.035	4.811	1.51E-06	0.038	4.682	2.87E-06	0.045	5.298	1.18E-07	··	··	··
PTR	R	0.034	4.680	2.89E-06	0.033	4.587	4.52E-06	0.050	5.826	5.79E-09	··	··	··
STR	L	0.037	5.102	3.39E-07	0.038	5.224	1.77E-07	0.055	6.453	1.13E-10	··	··	··
STR	R	··	··	··	··	··	··	0.044	5.129	2.95E-07	··	··	··
Fmin	R	··	··	··	··	··	··	0.034	3.788	1.53E-04	··	··	··
MCP		0.030	3.889	1.01E-04	0.032	4.232	2.33E-05	0.034	3.764	1.68E-04	··	··	··

Note. Reported β values are standardized. L/R: left/right, *t*: t-value; *p*: p-value. ATR, anterior thalamic radiation; BMI, body mass index; CingG, cingulum gyrus; EA, educational attainment, FA, fractional anisotropy; MD, mean diffusivity; Fmin, forceps minor; ILF, inferior longitudinal fasciculus; MCP, middle cerebellar peduncle; PTR, posterior thalamic radiation; SLF, superior longitudinal fasciculus; STR, superior thalamic radiation. Model A (n=17,975) estimated a linear regression with the IDPs as dependent variables and alcohol intake as the main independent variable of interest, controlling for sex, age, age-squared, age-cubed, height, brain volume, the Townsend index of social deprivation measured at the zip code level, handedness (right/left/ambidextrous; dummy-coded), the first 40 genetic principal components,[1] and dummy-coded county of residence[2] ($p < 2.25 \times 10^{-4}$). All continuous variables (except age-related variables) were standardized to a mean of 0 and a standard deviation of 1. Model B (n=17,975) included additional control for variables that are potentially associated with alcohol intake: weight, body mass index, and educational attainment ($p < 2.22 \times 10^{-4}$). Model C (n=16,606) repeated model A, excluding non-drinkers ($p < 1.97 \times 10^{-4}$). Model D (n=16,873) repeated model A, excluding heavy drinkers (i.e., women who reported consuming more than 18 units/week and men who reported consuming more than 24 units/week[2] ($p < 7.25 \times 10^{-4}$).

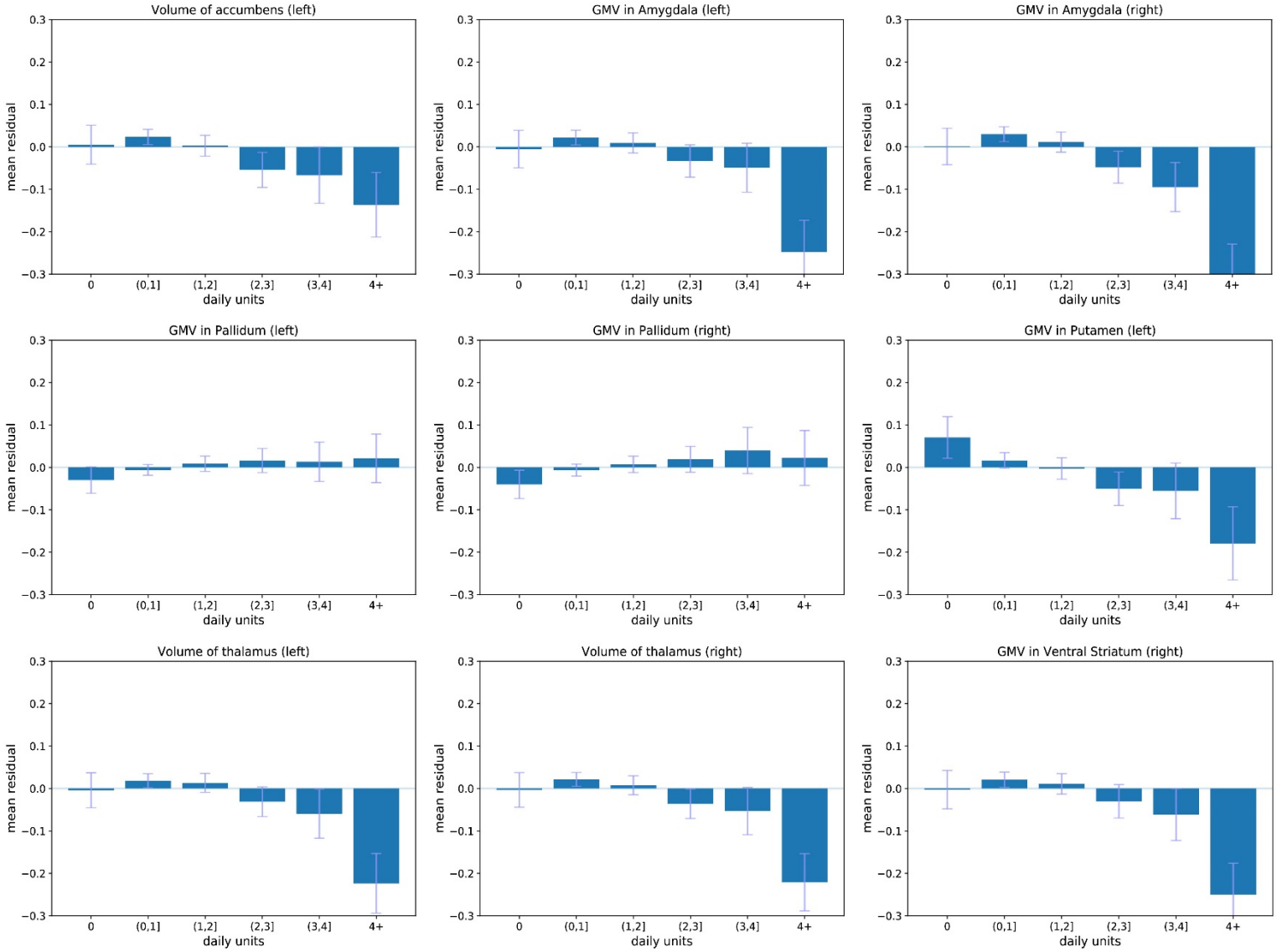
Supplementary Table 3. Associations between alcohol intake and neurite orientation dispersion and density imaging characteristics

		Model A			Model B			Model C			Model D		
Tract	**L/R**	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***	**β**	***t***	***p***
Intracellular Volume Fraction (ICVF)													
CingG	R	-0.030	-3.964	7.39E-05	-0.030	-3.979	6.94E-05	-0.035	-3.892	9.99E-05	··	··	··
IFOF	L	-0.032	-4.223	2.42E-05	-0.032	-4.220	2.46E-05	-0.037	-4.144	3.42E-05	··	··	··
IFOF	R	-0.033	-4.414	1.02E-05	-0.033	-4.348	1.38E-05	-0.038	-4.256	2.10E-05	··	··	··
ILF	L	··	··	··	··	··	··	-0.034	-3.724	1.97E-04	··	··	··
Unc	R	-0.029	-3.904	9.48E-05	-0.028	-3.766	1.67E-04	-0.034	-3.874	1.08E-04	··	··	··
ATR	L	··	··	··	-0.027	-3.711	2.07E-04	-0.035	-4.076	4.60E-05	··	··	··
ATR	R	··	··	··	-0.028	-3.888	1.01E-04	-0.036	-4.228	2.37E-05	··	··	··
PTR	L	-0.034	-4.538	5.71E-06	-0.035	-4.659	3.19E-06	-0.042	-4.705	2.56E-06	··	··	··
PTR	R	-0.034	-4.433	9.36E-06	-0.034	-4.433	9.37E-06	-0.045	-5.000	5.79E-07	··	··	··
STR	L	-0.035	-4.632	3.65E-06	-0.035	-4.593	4.40E-06	-0.046	-5.182	2.22E-07	··	··	··
STR	R	-0.031	-4.164	3.14E-05	-0.031	-4.095	4.23E-05	-0.044	-4.963	7.01E-07	··	··	··
Fmin		-0.037	-4.906	9.38E-07	-0.036	-4.823	1.43E-06	-0.041	-4.602	4.22E-06	··	··	··
Isotropic Volume Fraction (ISOVF)													
ILF	R	··	··	··	··	··	··	0.039	4.488	7.23E-06	··	··	··
SLF	R	··	··	··	··	··	··	0.035	4.036	5.45E-05	··	··	··
ATR	L	··	··	··	··	··	··	0.035	4.200	2.69E-05	··	··	··
PTR	L	0.037	5.145	2.70E-07	0.036	4.882	1.06E-06	0.047	5.539	3.27E-08	··	··	··
PTR	R	0.036	4.949	7.51E-07	0.035	4.848	1.26E-06	0.051	5.974	2.36E-09	··	··	··
STR	L	··	··	··	··	··	··	0.041	4.696	2.68E-06	··	··	··
MCP		0.034	4.426	9.65E-06	0.037	4.915	8.94E-07	0.040	4.445	8.86E-06	··	··	··
Tract Complexity/Fanning (OD)													
IFOF	L	··	··	··	··	··	··	-0.036	-3.884	1.03E-04	-0.030	-3.522	4.29E-04
IFOF	R	-0.031	-4.047	5.22E-05	-0.032	-4.115	3.89E-05	-0.037	-4.050	5.14E-05	-0.029	-3.497	4.71E-04
SLF	L	-0.035	-4.476	7.65E-06	-0.035	-4.522	6.16E-06	-0.040	-4.395	1.11E-05	··	··	··
SLF	R	-0.030	-3.912	9.16E-05	-0.031	-4.096	4.22E-05	-0.036	-4.030	5.60E-05	··	··	··
STR	L	-0.027	-3.718	2.02E-04	-0.027	-3.787	1.53E-04	-0.033	-3.836	1.25E-04	-0.033	-3.901	9.62E-05
STR	R	-0.032	-4.486	7.32E-06	-0.033	-4.531	5.92E-06	-0.042	-4.836	1.34E-06	··	··	··
AR	R	··	··	··	··	··	··	-0.039	-4.330	1.50E-05	··	··	··
CST	L	-0.041	-5.517	3.50E-08	-0.044	-5.861	4.69E-09	-0.049	-5.484	4.22E-08	-0.038	-4.665	3.11E-06
CST	R	-0.049	-6.504	8.01E-11	-0.052	-6.906	5.14E-12	-0.053	-5.983	2.23E-09	··	··	··
Fmaj		··	··	··	··	··	··	-0.042	-4.688	2.78E-06	··	··	··
Fmin		-0.032	-4.235	2.30E-05	-0.033	-4.447	8.76E-06	··	··	··	··	··	··

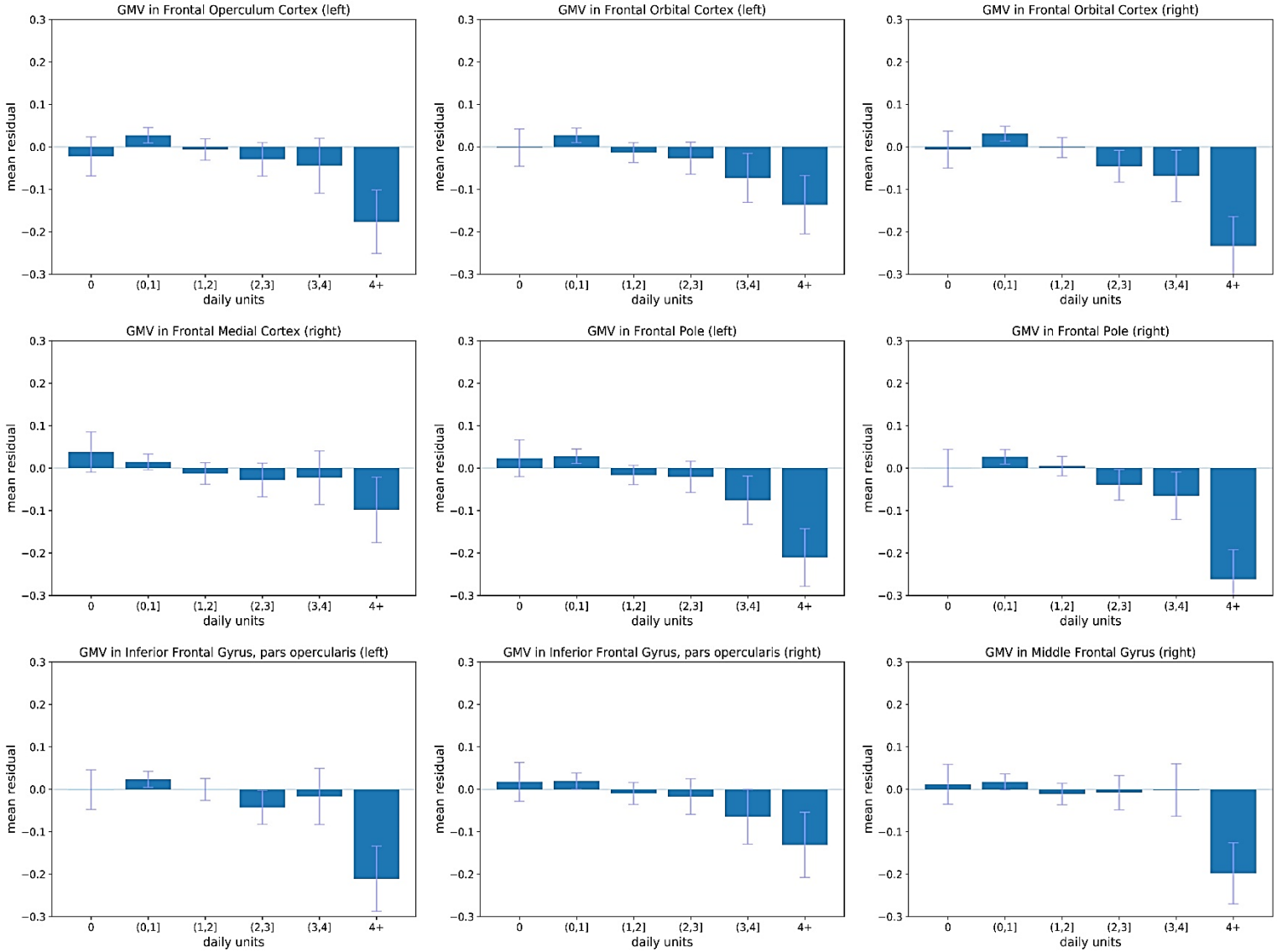
Note. Reported β values are standardized. L/R: left/right, *t*: t-value; *p*: p-value. ATR, anterior thalamic radiation; BMI, body mass index; CingG, cingulum gyrus; EA, educational attainment, FA, fractional anisotropy; MD, mean diffusivity; Fmin, forceps minor; ILF, inferior longitudinal fasciculus; MCP, middle cerebellar peduncle; PTR, posterior thalamic radiation; SLF, superior longitudinal fasciculus; STR, superior thalamic

radiation. Model A (n=17,975) estimated a linear regression with the IDPs as dependent variables and alcohol intake as the main independent variable of interest, controlling for sex, age, age-squared, age-cubed, height, brain volume, the Townsend index of social deprivation measured at the zip code level, handedness (right/left/ambidextrous; dummy-coded), the first 40 genetic principal components,[1] and dummy-coded county of residence[2] ($p < 2.25 \times 10^{-4}$). All continuous variables (except age-related variables) were standardized to a mean of 0 and a standard deviation of 1. Model B (n=17,975) included additional control for variables that are potentially associated with alcohol intake: weight, body mass index, and educational attainment ($p < 2.22 \times 10^{-4}$). Model C (n=16,606) repeated model A, excluding non-drinkers ($p < 1.97 \times 10^{-4}$). Model D (n=16,873) repeated model A, excluding heavy drinkers (i.e., women who reported consuming more than 18 units/week and men who reported consuming more than 24 units/week[3] ($p < 7.25 \times 10^{-4}$).

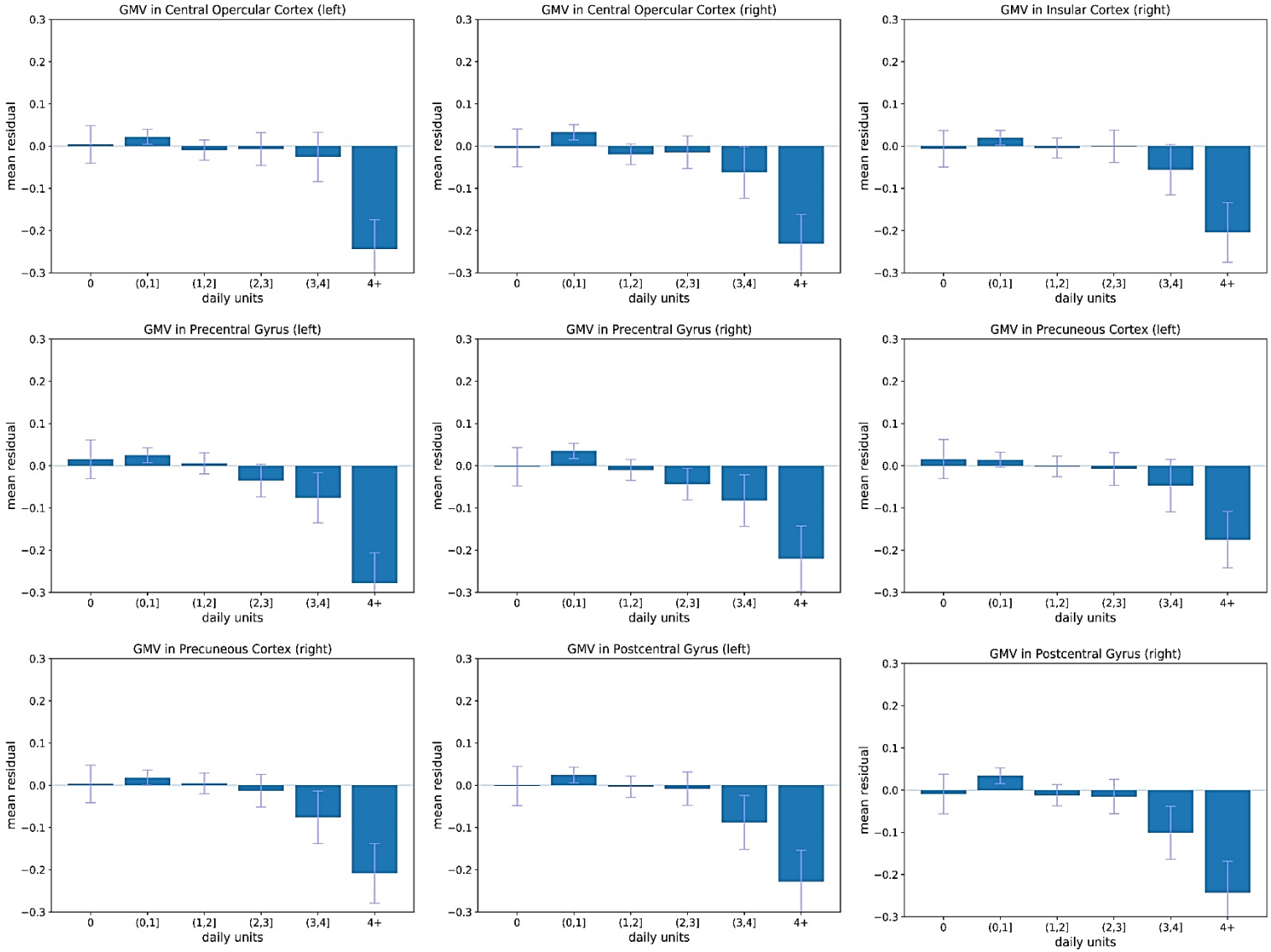
Supplementary Figure 1. Average regional gray matter volume in subcortical brain regions showing significant associations from Model A (linear regression) based on daily drinking levels. Daily unit = 10 ml of pure ethanol.



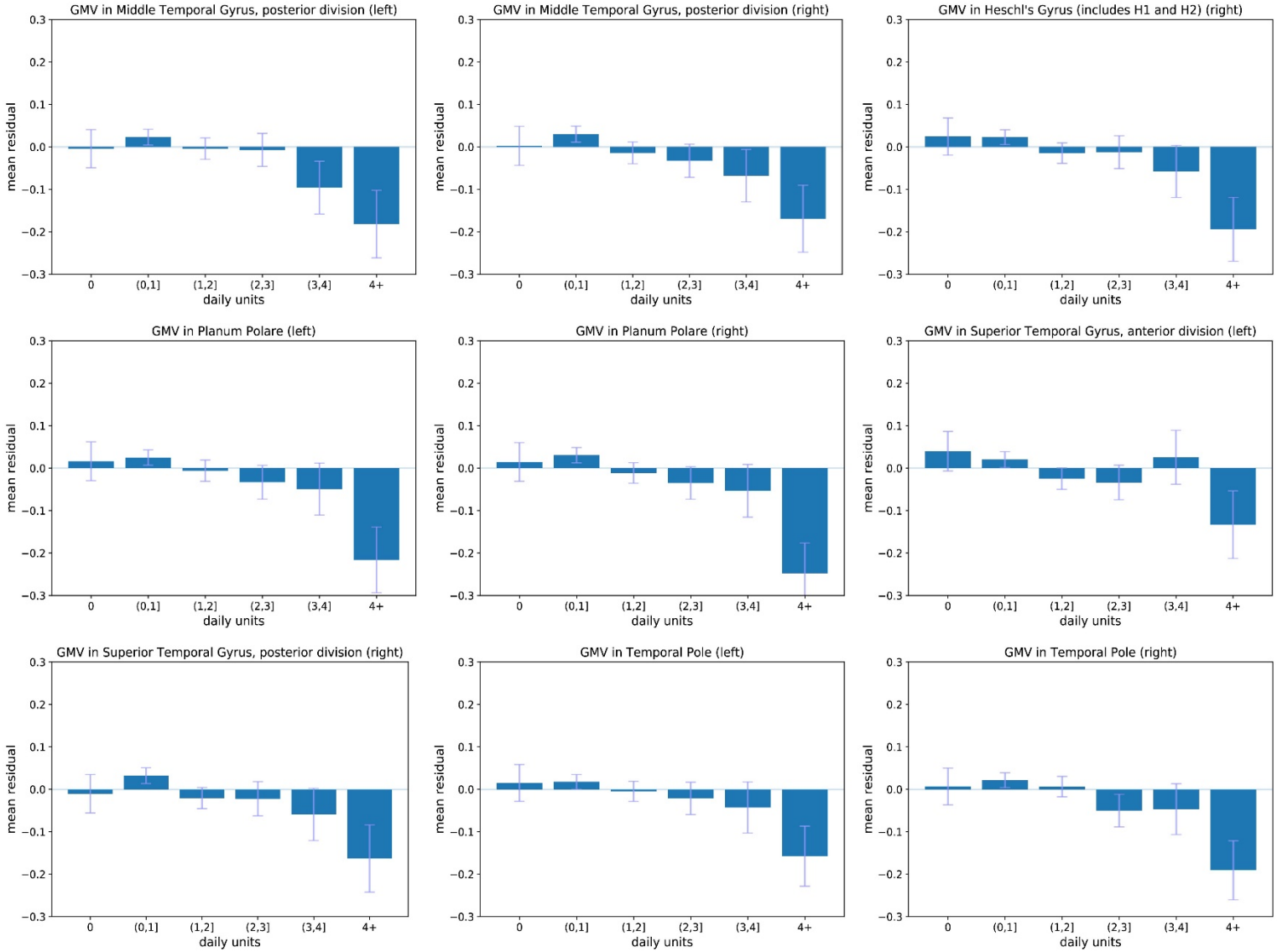
Supplementary Figure 2. Average regional gray matter volume in frontal brain regionals showing significant associations in Model A (linear regression) across average daily drinking levels. Daily unit = 10 ml of pure ethanol.



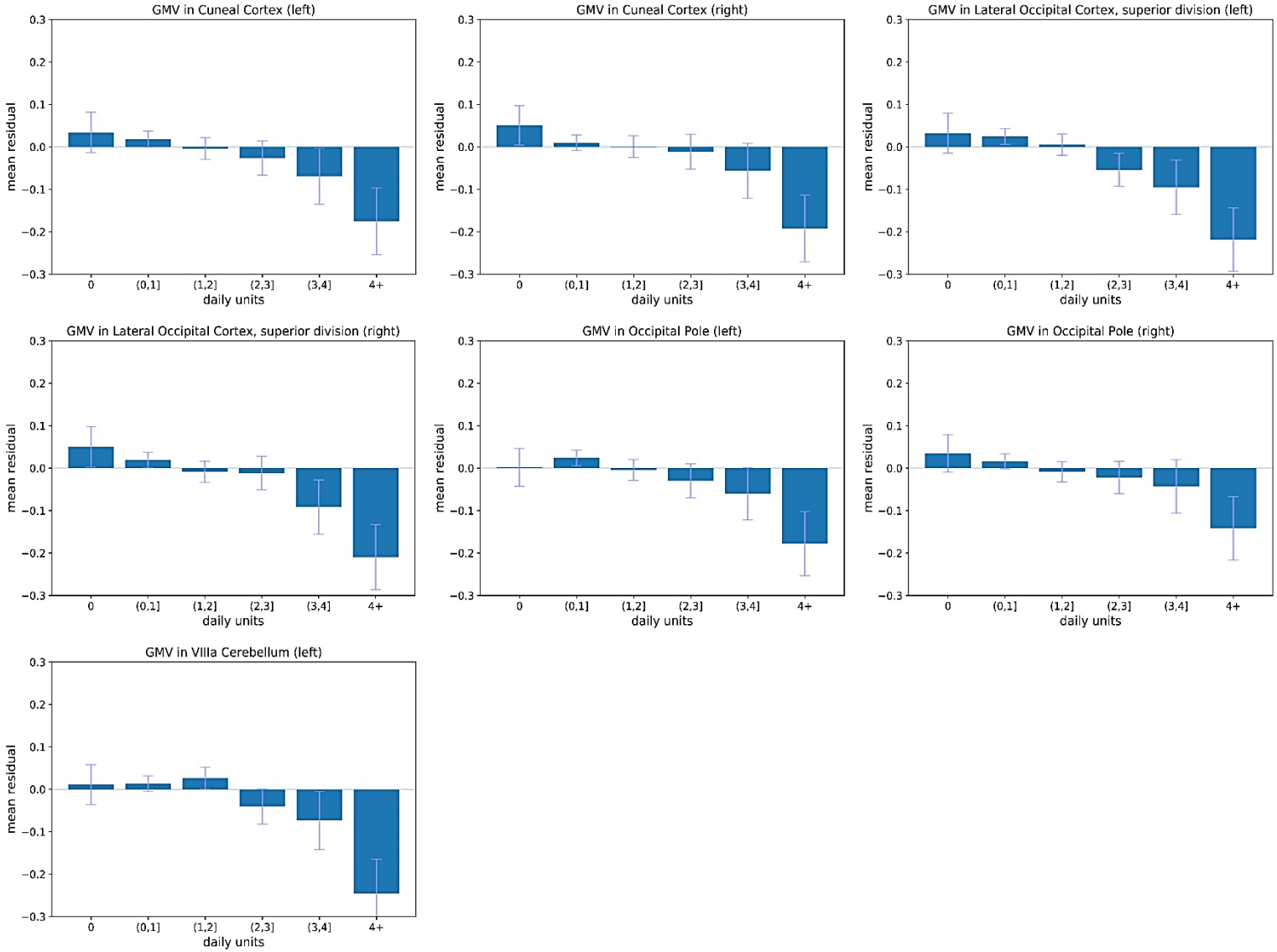
Supplementary Figure 3. Average regional gray matter volume in frontal, insular, and parietal brain regions showing significant associations from Model A (linear regression) across average daily drinking levels. Daily unit = 10 ml of pure ethanol.



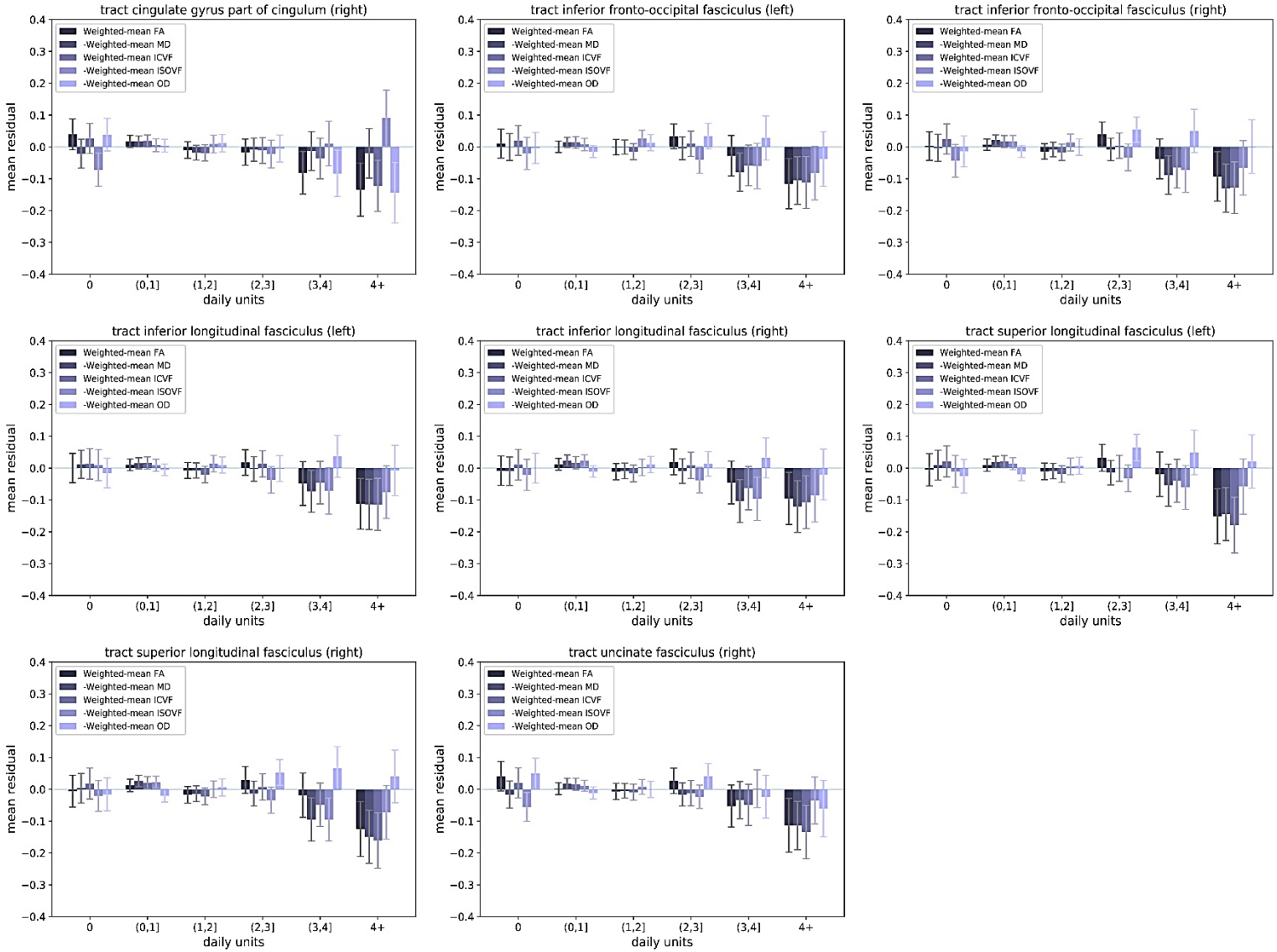
Supplementary Figure 4. Average regional gray matter volume in temporal brain regions showing significant associations in Model A (linear regression) across average daily drinking levels. Daily unit = 10 ml of pure ethanol.



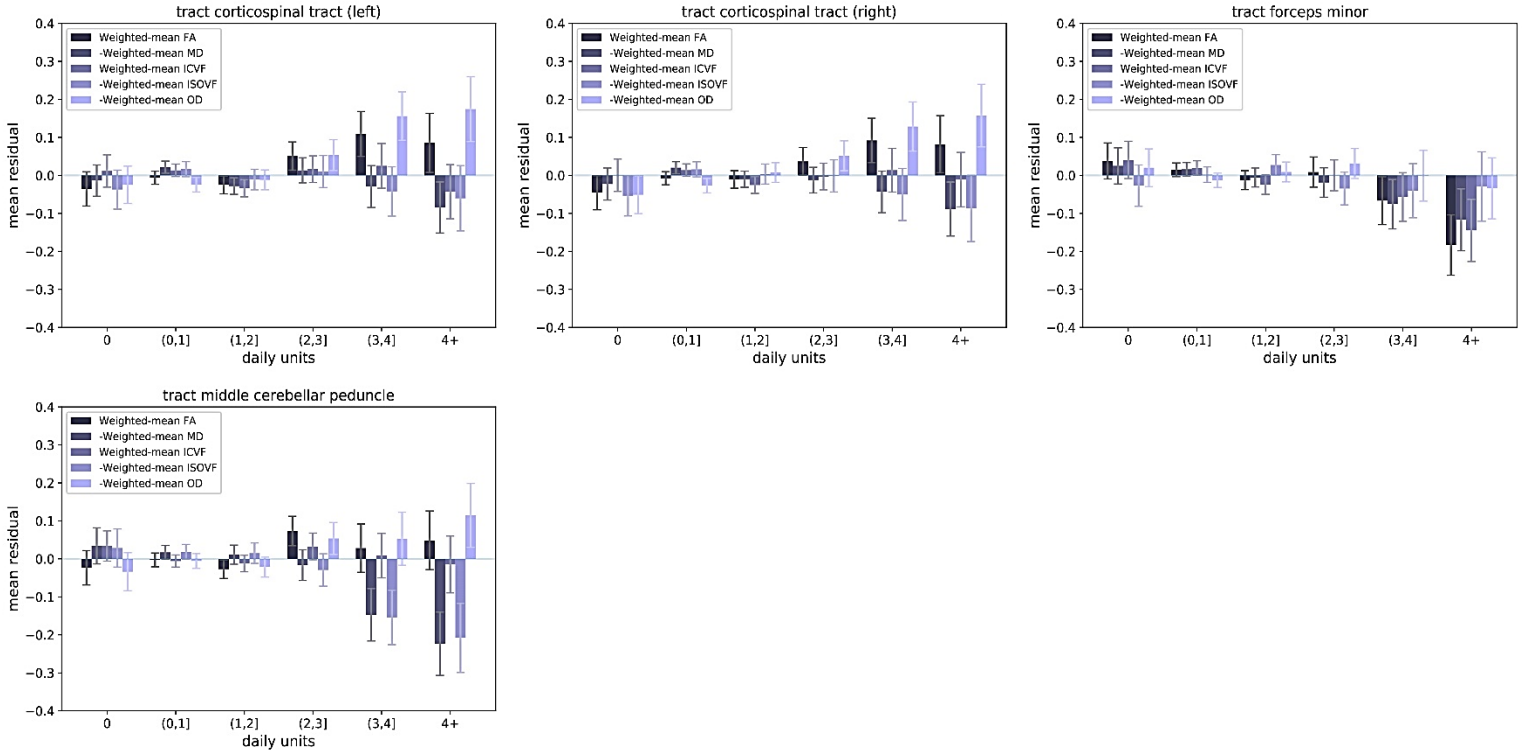
Supplementary Figure 5. Average regional gray matter volume in occipital and cerebellar brain regions showing significant associations from Model A (linear regression) across average daily drinking levels. Daily unit = 10 ml of pure ethanol.



Supplementary Figure 6. Average white matter microstructure indices showing significant associations from Model A (linear regression) across average daily drinking levels in association fibers. Daily unit = 10 ml of pure ethanol.



Supplementary Figure 7. Average white matter microstructure indices showing significant associations from Model 1 (linear regression) across average daily drinking levels in projection fibers. Daily unit = 10 ml of pure ethanol.



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