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Investigating the causal nature of the relationship of subcortical brain volume with smoking and alcohol use

Emma Logtenberg*, Martin F. Overbeek*, Joëlle A. Pasman, Abdel Abdellaoui, Maartje Luijten, Ruth J. van Holst, Jacqueline M. Vink, Damiaan Denys, Sarah E. Medland, Karin J. H. Verweij and Jorien L. Treur

Background

Structural variation in subcortical brain regions has been linked to substance use, including the most commonly used substances nicotine and alcohol. Pre-existing differences in subcortical brain volume may affect smoking and alcohol use, but there is also evidence that smoking and alcohol use can lead to structural changes.

Aims

We assess the causal nature of the complex relationship of subcortical brain volume with smoking and alcohol use, using bi-directional Mendelian randomisation.

Method

Mendelian randomisation uses genetic variants predictive of a certain 'exposure' as instrumental variables to test causal effects on an 'outcome'. Because of random assortment at meiosis, genetic variants should not be associated with confounders, allowing less biased causal inference. We used summary-level data of genome-wide association studies of subcortical brain volumes (nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen and thalamus; $n = 50\,290$) and smoking and alcohol use (smoking initiation, $n = 848\,460$; cigarettes per day, $n = 216\,590$; smoking cessation, $n = 378\,249$; alcoholic drinks per week, $n = 630\,154$; alcohol dependence, $n = 46\,568$). The main analysis, inverse-variance weighted regression, was verified by a wide range of sensitivity methods.

Results

There was strong evidence that liability to alcohol dependence decreased amygdala and hippocampal volume, and smoking more cigarettes per day decreased hippocampal volume. From subcortical brain volumes to substance use, there was no or weak evidence for causal effects.

Conclusions

Our findings suggest that heavy alcohol use and smoking can causally reduce subcortical brain volume. This adds to accumulating evidence that alcohol and smoking affect the brain, and likely mental health, warranting more recognition in public health efforts.

Keywords

Smoking; alcohol use; alcohol dependence; subcortical brain volume; Mendelian randomisation.

Subcortical brain regions have consistently been implicated in substance use, playing a crucial role in the brain's reward system. Addiction is thought to reflect a vicious cycle of intoxication, withdrawal and craving, with (subcortical) brain circuits mediating these stages.[1] The causal nature of the relationship between structural variation in subcortical brain regions and substance use is unclear. Subcortical brain volume and substance use are heritable, and there is evidence that they share (part of) their genetic aetiology.[2] Alternatively, the relationship may be causal, with pre-existing differences in subcortical brain volume asserting a direct effect on substance use. Causality in the other direction is also plausible, i.e. substance use affecting brain structure. Most likely, the relationship between subcortical brain volume and substance use reflects a combination of mechanisms.

The majority of substance use-related morbidity and mortality result from smoking and alcohol use.[3] Literature on the relationship of subcortical brain volumes with smoking and alcohol use comprises predominantly small cross-sectional studies, reporting mixed findings. Smoking has been associated with smaller nucleus accumbens,[4] amygdala,[5,6] hippocampus,[7] pallidum[6] and thalamus[6] volumes, with smaller[5] and larger[8] caudate volume, and larger putamen volume.[4] Alcohol use has been associated with smaller nucleus accumbens,[9] amygdala,[10] hippocampus[9] and pallidum volumes,[9] and with smaller[11] and larger[12] caudate, smaller[9] and larger[13] thalamus, and smaller[9] and larger[13] putamen volumes. Recently, the Enhancing Neuro Imaging Genetics Through Meta Analysis (ENIGMA) addiction working group attempted to resolve these inconsistent findings with a mega-analysis of subcortical thickness and surface area, among 1628 controls and 2277 individuals dependent on alcohol, nicotine, cocaine, methamphetamine and/or cannabis.[14] Smoking was associated with greater thickness and surface area, most notably for the nucleus accumbens and hippocampus, whereas alcohol dependence was associated with lower thickness and surface area, most notably for the hippocampus, amygdala, thalamus and putamen.

Identifying causal relationships

Longitudinal studies investigating subcortical brain volume and substance use are scarce. One recent study in 714 individuals at ages 14 and 19 years showed that alcohol and cannabis use were associated with accelerated cortical thinning and a (mild) increase in subcortical volumes.[15] Although these were longitudinal analyses, the observational design leaves potential for bias from

* Joint first authorship.

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(unmeasured) confounding and reverse causality. A randomised controlled trial would be unfeasible for the relationship under study, but Mendelian randomisation provides an alternative.[16] Instead of experimental manipulation, Mendelian randomisation uses genetic variants as proxies for the independent variable. Because genes are randomly transmitted from parents to offspring, genetic variants should not be associated with confounders. Reverse causation is not likely either, as the genetic independent variable is fixed at birth. A recent Mendelian randomisation study found no evidence for a causal effect of smoking on hippocampal volume,[17] but the analyses were based on much smaller samples than currently available and other subcortical regions were not included. We conduct the first comprehensive Mendelian randomisation study, using the largest genetic data-sets available of the volume of seven subcortical regions (nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus) and substance use (smoking initiation, cigarettes per day, smoking cessation, alcoholic drinks per week and alcohol dependence), to probe bi-directional, causal relationships.

Method

Mendelian randomisation

Mendelian randomisation is based on the premise that genetic markers can be used as proxies for a variable hypothesised to be a risk factor, or 'exposure', for another 'outcome' variable. Single nucleotide polymorphisms (SNPs) are most often used. The validity of Mendelian randomisation relies on three assumptions: the association of the genetic instrument with the exposure is robust (ensured by selecting SNPs under $P < 5e{-}08$); the instrument is not associated with confounding variables and the instrument does not influence the outcome through any other path than the exposure. Horizontal pleiotropy, where an SNP directly affects multiple traits, could lead to the second and third assumptions being violated. To assess whether the assumptions were met, we applied a wide range of sensitivity methods.

Data

All data-sets and measures used for our analyses are visually depicted in Supplementary Fig. 1 available at https://doi.org/10.1192/bjp.2021.81, and described in more detail in Supplementary Table 1. For subcortical brain volumes, we used summary-level data from a published genome-wide association study (GWAS) ($n = 13\,171$[18]), and meta-analysed these with summary-level data from a GWAS of 37 119 UK Biobank participants (Supplementary Appendix 1). This resulted in a sample of 50 290 for nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus volumes. For substance use, we obtained summary-level data from the single largest GWAS on smoking and alcohol use.[19] To prevent sample overlap, we excluded UK Biobank data from this GWAS. We meta-analysed the remaining summary statistics with those from 23andMe (separately obtained from 23andMe, as these are not publicly available), resulting in sample sizes of $n = 848\,460$ for smoking initiation, $n = 216\,590$ for cigarettes per day, $n = 378\,249$ for smoking cessation and $n = 630\,154$ for alcoholic drinks per week. We also obtained summary-level data from a separate GWAS on alcohol dependence ($n = 46\,568$).[20] Both the subcortical brain volumes and substance use meta-analyses were *N*-weighted, owing to measurement variance in the original samples, resulting in *z*-scores. To allow Mendelian randomisation analysis, we constructed beta-coefficients and standard errors using these *z*-scores, effect allele frequencies and sample size.[21] Although the unit of Mendelian randomisation estimates based on constructed beta-coefficients and s.e. cannot be reliably interpreted, the direction of effect and statistical strength is robust.

Because we obtained the exposure and outcome estimates from separate samples, it is not possible to verify if individuals in the outcome sample were affected by the exposure. Therefore, when we refer to an exposure causally affecting an outcome, this should be interpreted as an effect of the liability to that exposure. We assessed smoking and alcohol use in 37 119 UK Biobank participants included in the subcortical brain volume GWAS, to confirm that smoking and alcohol use were prevalent (Supplementary Table 2).

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Summary-level data from consortia were used, with individual sites having obtained ethical approval and informed consent from participants. For UK Biobank (http://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=200), the National Health Service North West Centre for Research Ethics Committee provided ethical approval (reference umber 11/NW/0382).

Main analysis

We explain the analysis by using an example where hippocampal volume is the exposure (the independent variable) and smoking initiation is the outcome (the dependent variable). First, SNPs that robustly predict hippocampal volume ($P < 5E{-}08$) were identified in the hippocampus GWAS, and their effect size estimates and standard errors were extracted. These SNPs – the genetic instrument – were then identified in the smoking initiation GWAS, and their effect estimates and standard errors were extracted. To estimate the causal effect, the SNP–smoking initiation association was divided by the SNP–hippocampal volume association for each SNP separately. To reach a combination of effects with minimum variance for all SNPs combined, the individual SNP effects were weighted by the inverse of their variance. Inverse-variance weighted regression (IVW) provides the first indication of causality, assuming that all Mendelian randomisation assumptions are met.

We tested causal relationships with subcortical brain volumes as exposures and smoking initiation, cigarettes per day, smoking cessation, alcoholic drinks per week and alcohol dependence as outcomes, and *vice versa*, with smoking initiation, alcoholic drinks per week and alcohol dependence as exposures and subcortical volumes as outcomes. If fewer than ten SNPs with $P < 5e{-}08$ were available, we additionally constructed an instrument containing SNPs under a more lenient threshold of $P < 1e{-}05$. We clumped SNPs for independence at $r^2 < 0.01$ and 10 000 kb.[22]

Because the GWAS for cigarettes per day consisted of smokers only,[19] SNPs from that study cannot be used as proxies for cigarettes per day in never smokers. Therefore, the complete subcortical brain volume data-set ($n = 50\,290$), consisting of smokers and never smokers, could not be used. For UK Biobank participants ($n = 37\,119$), we could obtain information on smoking and perform GWAS of subcortical brain volumes in never smokers ($n = 22\,555$) and ever smokers ($n = 14\,564$). We then applied summary-level Mendelian randomisation with cigarettes per day as the exposure, in never and ever smokers separately. If the genetic instrument for cigarettes per day predicts subcortical brain volume in never smokers, this indicates horizontal pleiotropy because there cannot be a true causal effect.[23] Note that a similar stratification approach was not indicated for alcohol use, as the exposure sample from which the genetic instruments for alcoholic drinks per week were obtained included non-drinkers. Unlike for smoking, almost all participants in the European samples used are expected to have been exposed to (some) alcohol. Also note

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that all GWAS included in our analyses corrected for age and gender (Supplementary Table 2).

Sensitivity analyses

The *F*-statistic was computed to assess instrument strength for all exposures, with $F > 10$ reflecting a sufficiently strong instrument.[24] To test the robustness of IVW, we applied six sensitivity methods with different, partly contrasting assumptions. First, we used weighted median regression, which produces a reliable causal estimate if ≥50% of the total weight of the genetic instrument comes from valid (unbiased) SNPs.[25] Second, we used weighted mode regression, which clusters SNPs in the genetic instrument and selects the SNP cluster with the largest weight as the final causal estimate. This results in an unbiased value if the most common causal estimate is indeed the true causal effect.[26] Third, we used Mendelian randomisation-Egger (MR-Egger), which permits the intercept to deviate from zero, allowing a formal test of horizontal pleiotropy (when there is no horizontal pleiotropy, the intercept is zero).[27] MR-Egger is reliable if the instrument strength independent of direct effect (InSIDE) assumption is met, meaning that the strength of the instrument (SNP–exposure association) should not correlate with the direct effect of the SNPs on the outcome. MR-Egger also requires sufficiently strong instruments, indicated as the no measurement error (NOME) assumption. This can be assessed with the IGX2 statistic, ranging between 0 and 1. A lower value reflects a higher chance that NOME is violated.[28] If IGX2 ≥ 0.9, NOME is unlikely to be violated and results are reliable. If IGX2 is within 0.6–0.9, NOME may have been violated but MR-Egger simulation extrapolation (SIMEX) can correct for this. If IGX2 < 0.6, MR-Egger results are likely biased. Fourth, we used generalised summary-data-based Mendelian randomisation (GSMR), which accounts for very low levels of linkage disequilibrium between SNPs and sampling variance in the estimated SNP effects, to attain higher statistical power. GSMR filters outlier SNPs based on their effect size (heterogeneity in dependent instruments (HEIDI) filtering).[29] Fifth, we used Mendelian randomisation pleiotropy residual sum and outlier (MR-PRESSO), which compares the observed residual sum of squares with the expected residual sum of squares for each SNP, re-running outlier-corrected IVW analyses.[30] Sixth, we used Steiger filtering, which identifies potential bias from reverse causation. It calculates how much variance each SNP explains in the exposure and the outcome, and tests whether the explained variance is, as expected, higher for exposure than outcome. SNPs that explain more variance in the outcome than the exposure are excluded.[31]

We computed Cochran's *Q*-statistic to assess heterogeneity across the causal estimates of SNPs in each instrument[24]; high heterogeneity points to horizontal pleiotropy. It is also possible for a true causal effect to run through multiple, separate biological pathways, resulting in heterogeneity. To assess variability in the power of the genetic instruments, we computed the amount of variance that each instrument explained in the proposed exposure variable.[32]

Analyses were conducted in R version 4.0.3 for Windows (see see https://cran.r-project.org/bin/windows/base/old/4.0.3/), using TwoSampleMR,[22] GSMR[29] and MR-PRESSO[30] packages.

Appraisal of the evidence

We did not correct for multiple testing explicitly because we analyse phenotypes for which, *a priori*, there are plausible hypotheses why they are (causally) associated and we want to avoid appraising evidence based on an arbitrary threshold. Rather than using a strict significance threshold, we ascribe a finding as showing strong evidence, evidence, weak evidence or no clear evidence for a causal effect, based on both the IVW result (adhering to the interpretation of *P*-values suggested by Sterne et al,[33] who stressed the importance of considering *P*-values and evidence on a spectrum, rather than in discrete terms) and the sensitivity analyses. This approach provides a safeguard against chance findings, since a finding is only considered robust when the main result is corroborated by sensitivity analyses. A similar approach has previously been adopted by other, high-impact Mendelian randomisation studies.[34,35] Note that because sensitivity methods rely on stricter assumptions than IVW, their statistical power is lower. It is to be expected that the statistical evidence, but not the effect size, decreases with stricter sensitivity methods, even for a true causal effect.

Results

All genetic instruments showed sufficient strength (Supplementary Tables 3–5). The amount of variance that the genetic instruments explained in the corresponding subcortical region and substance use variables, as well as the estimated SNP heritability for each variable are presented in Table 1. Figure 1 displays all relationships with (weak-to-strong) evidence for causality.

Table 1 Explained variance for the genetic instruments used for Mendelian randomisation analyses and SNP-based heritability estimates for all phenotypes, based on full summary statistics

Variable	Threshold	Number of SNPs in genetic instrument	Percentage of variance that the genetic instrument explains in the variable	SNP-based heritability
Nucleus accumbens	5.00E−08	13	0.70%	16.70%[a]
Amygdala	5.00E−08	2	0.17%	9.26%[a]
Amygdala	1.00E−05	40	0.97%	9.26%
Caudate	5.00E−08	53	3.97%	31.66%[a]
Hippocampus	5.00E−08	19	1.60%	17.24%[a]
Pallidum	5.00E−08	25	1.82%	18.44%[a]
Putamen	5.00E−08	35	2.18%	27.65%[a]
Thalamus	5.00E−08	12	0.70%	20.63%[a]
Smoking initiation	5.00E−08	346	1.43%	7.77%[b]
Cigarettes per day	5.00E−08	49	1.27%	8.04%[b]
Alcoholic drinks per week	5.00E−08	92	0.56%	4.19%[b]
Alcohol dependence	5.00E−08	10	0.59%	9.00%[b]

Explained variance was computed as described by Pasman et al.[32] SNP, single nucleotide polymorphism.
a. SNP-based heritability calculated by us.
b. SNP-based heritability as reported by Liu et al.[19]

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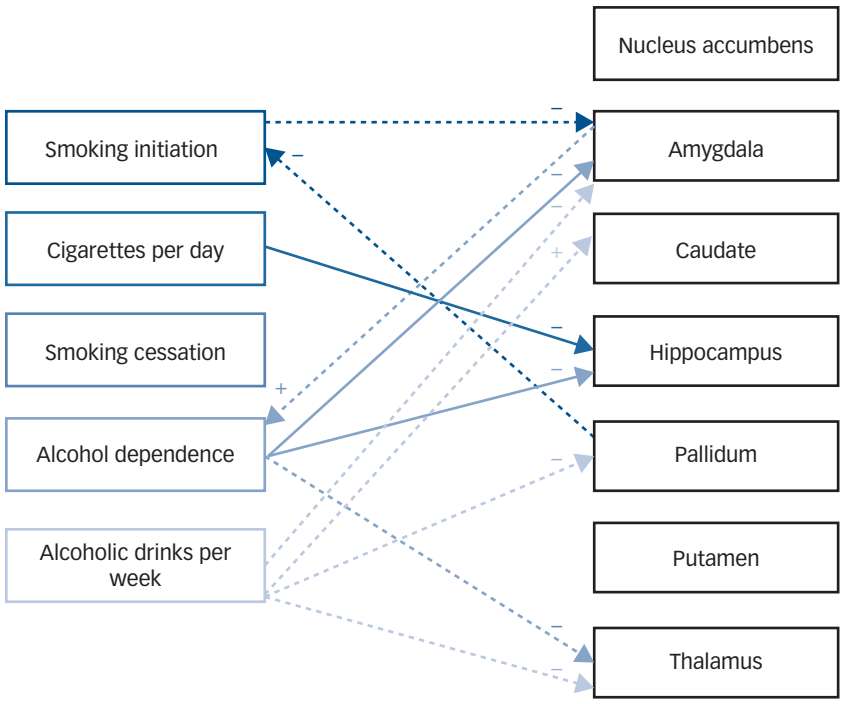

Fig. 1 Graphical display of the relationships with evidence for causality. Minus (–) signifies a negative, decreasing effect; plus (+) signifies a positive, increasing effect. The thicker lines reflect evidence or strong evidence for causality; the dotted, thinner lines signify weak evidence for causality. Note that causal effects were tested in both directions for all relationships except for smoking cessation, which was only tested as an outcome variable.

Causal relationships from subcortical volumes to substance use

There was weak evidence that a larger pallidum volume decreases the odds of initiating smoking ($\beta_{IVW} = -0.04$, $P = 0.053$). Weighted median, weighted mode and GSMR corroborated this, showing similar effect sizes and stronger statistical evidence (Table 2). Although there was no clear evidence for horizontal pleiotropy (MR-Egger intercept −0.003, $P = 0.332$; Supplementary Table 6), the regression coefficient of MR-Egger did not indicate a causal effect (Table 2). There was strong evidence for heterogeneity among the SNP effects (Cochran's Q-statistic $P = 2.4E{-}05$; Supplementary Table 7). MR-PRESSO identified two SNP outliers but there was no distortion of the causal estimate after outlier removal (Supplementary Table 8). Steiger filtering did not identify SNPs that explained more variance in the outcome than the exposure (Supplementary Table 9).

There was weak evidence that a larger amygdala volume increases alcohol dependence risk ($P < 1E{-}05$, $\beta_{IVW} = 0.08$, $P = 0.046$), corroborated by weighted median, weighted mode and GSMR, but not MR-Egger. There was no clear evidence for horizontal pleiotropy (MR-Egger intercept 0.003, $P = 0.400$) or heterogeneity ($P = 0.621$). MR-PRESSO did not identify SNP outliers, nor did Steiger filtering identify SNPs that explained more variance in the outcome than the exposure. With a two-SNP instrument ($P < 5E{-}08$), there was a similar sized positive effect, but no clear statistical evidence ($\beta_{IVW} = 0.09$, $P = 0.522$).

There was very weak evidence that a larger amygdala volume increased the number of alcoholic drinks per week ($\beta_{IVW} = 0.06$, $P = 0.098$), but sensitivity analyses were not possible because the $P < 5E{-}08$ instrument only contained two SNPs. With 40 SNPs under $P < 1E{-}05$, there was no clear evidence for an effect ($\beta_{IVW} = 0.01$, $P = 0.289$).

No other analyses showed clear evidence for causal effects.

Causal relationships from substance use to subcortical volumes

There was weak evidence that smoking initiation decreases amygdala volume ($\beta_{IVW} = -0.05$, $P = 0.046$), confirmed by GSMR (Table 3). There was no clear evidence for horizontal pleiotropy (MR-Egger intercept −0.001, $P = 0.457$), but strong evidence for heterogeneity (Cochran's Q-statistic $P = 2.4E{-}07$). MR-PRESSO identified one SNP outlier, which did not affect the results. Steiger filtering excluded 44 SNPs, but after running the analyses with the 302 remaining SNPs, evidence for a causal effect remained ($\beta_{IVW} = -0.06$, $P = 0.013$).

In the analyses stratified for smoking status, there was strong evidence that smoking more cigarettes per day decreases hippocampal volume in smokers ($\beta_{IVW} = -94.73$, $P = 1.8E{-}06$). Results were consistent with weighted median, weighted mode, MR-Egger and GSMR, albeit with a smaller effect size for the latter. There was no clear evidence for horizontal pleiotropy (MR-Egger intercept 0.633, $P = 0.568$) or heterogeneity ($P = 0.357$). No SNP outliers were identified with MR-PRESSO. Steiger filtering identified nine SNPs, but after excluding these (leaving 40 SNPs), strong and consistent evidence for causality remained. There was weak evidence for a negative effect of cigarettes per day on hippocampal volume in never smokers (indicating horizontal pleiotropy), with a much smaller, less significant effect size ($\beta_{IVW} = -30.40$, $P = 0.050$) and no consistency across sensitivity methods. Since the effect size and statistical evidence were substantially larger and stronger in ever compared with never smokers (Supplementary Fig. 2), there is likely a negative causal effect of smoking heaviness on hippocampal volume (horizontal pleiotropy notwithstanding).

There was evidence for a negative effect of cigarettes per day on putamen volume, but this relationship seems pleiotropic. The effect size and statistical evidence in ever and never smokers is nearly identical ($\beta_{IVW} = -68.86$, $P = 0.018$ and $\beta_{IVW} = -71.41$, $P = 0.003$,

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Table 2 Mendelian randomisation analysis, with subcortical brain volumes as the exposures and smoking and alcohol use as the outcomes

		SNP	IVW			Weighted median			Weighted mode			MR-Egger[a]			SNP	GSMR		
Exposure	Outcome	*n*	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*n*	*β*-value	95% CI	*P*-value
Nucleus accumbens	Smoking initiation	13	0.02	−0.05 to 0.09	0.542	−0.01	−0.08 to 0.06	0.789	−4.3E−04	−0.12 to 0.12	0.994	−0.24	−0.37 to −0.11	0.003	13	0.02	−0.03 to 0.06	0.519
Amygdala $P < 5E{-}08$	Smoking initiation	2	0.10	−0.12 to 0.31	0.388	–	–	–	–	–	–	–	–	–	–	–	–	–
Amygdala $P < 1E{-}05$	Smoking initiation	40	−0.01	−0.04 to 0.03	0.770	3.6E−03	−0.04 to 0.04	0.864	1.0E−03	−0.04 to 0.04	0.957	0.04	−0.01 to 0.10	0.134	40	5.6E−04	−0.03 to 0.03	0.966
Caudate	Smoking initiation	53	−0.02	−0.05 to 0.02	0.298	−5.8E−03	−0.04 to 0.03	0.740	−2.1E−03	−0.05 to 0.04	0.931	0.05	−0.06 to 0.17	0.352	49	4.3E−03	−0.02 to 0.03	0.702
Hippocampus	Smoking initiation	19	0.02	−0.07 to 0.01	0.644	0.02	−0.05 to 0.09	0.545	0.04	−0.10 to 0.17	0.599	−0.30	−0.81 to 0.21	0.271	19	5.4E−04	−0.04 to 0.04	0.978
Pallidum	Smoking initiation	25	−0.04	−0.08 to 0.07E−04	0.053	−0.05	−0.09 to −0.01	0.024	−0.07	−0.12 to −0.03	0.006	1.8E−03	−0.09 to 0.09	0.970	23	−0.05	−0.07 to −0.02	0.001
Putamen	Smoking initiation	35	−3.8E−03	−0.05 to 0.04	0.859	−0.02	−0.05 to 0.01	0.200	−0.02	−0.05 to 0.01	0.266	0.02	−0.08 to 0.11	0.745	29	1.8E−03	−0.02 to 0.02	0.880
Thalamus	Smoking initiation	12	−0.05	−0.16 to 0.05	0.291	−0.06	−0.14 to 0.03	0.177	−0.06	−0.20 to 0.08	0.395	−0.16	−0.36 to 0.04	0.139	10	3.5E−03	−0.06 to 0.05	0.903
Nucleus accumbens	Smoking cessation	13	0.02	−0.02 to 0.06	0.307	0.02	−0.03 to 0.07	0.477	0.04	−0.03 to 0.11	0.304	0.06	−0.04 to 0.17	0.265	13	−0.02	−0.06 to 0.02	0.303
Amygdala $P < 5E{-}08$	Smoking cessation	2	−0.14	−0.32 to 0.04	0.131	–	–	–	–	–	–	–	–	–	–	–	–	–
Amygdala $P < 1E{-}05$	Smoking cessation	39	−0.01	−0.04 to 0.01	0.406	−0.03	−0.07 to 0.01	0.150	−0.02	−0.08 to 0.04	0.501	−0.07	−0.19 to 0.05	2.309	39	0.01	−0.01 to 0.04	0.348
Caudate	Smoking cessation	53	0.01	−0.02 to 0.04	0.706	−2.4E−03	−0.04 to 0.03	0.886	−0.01	−0.07 to 0.05	0.848	−8.5E−03	−0.09 to 0.07	0.831	50	−0.01	−0.03 to 0.01	0.389
Hippocampus	Smoking cessation	19	0.01	−0.04 to 0.06	0.586	−0.01	−0.06 to 0.04	0.742	−0.03	−0.12 to 0.06	0.523	−0.03	−0.27 to 0.21	0.800	19	−0.01	−0.05 to 0.02	0.528
Pallidum	Smoking cessation	25	0.02	−0.01 to 0.06	0.110	0.01	−0.03 to 0.05	0.560	0.01	−0.04 to 0.05	0.781	−0.02	−0.08 to 0.05	0.635	25	−0.02	−0.05 to 0.00	0.061
Putamen	Smoking cessation	35	0.01	−0.02 to 0.05	0.513	8.5E−03	−0.03 to 0.05	0.660	0.01	−0.05 to 0.06	0.797	−0.03	−0.12 to 0.06	0.532	35	−0.01	−0.03 to 0.01	0.355
Thalamus	Smoking cessation	13	3.7E−03	−0.06 to 0.07	0.907	4.3E−03	−0.06 to 0.06	0.889	0.01	−0.07 to 0.08	0.864	−0.10	−0.31 to 0.11	0.361	13	2.9E−03	−0.04 to 0.04	0.893
Nucleus accumbens	CPD	13	0.04	−0.01 to 0.09	0.111	0.04	−0.02 to 0.11	0.198	0.06	−0.05 to 0.16	0.312	0.07	−0.05 to 0.19	0.254	13	0.04	−0.01 to 0.09	0.129
Amygdala $P < 5E{-}08$	CPD	2	0.02	−0.11 to 0.16	0.741	–	–	–	–	–	–	–	–	–	–	–	–	–
Amygdala $P < 1E{-}05$	CPD	40	−0.01	−0.05 to 0.03	0.582	0.01	−0.04 to 0.06	0.770	0.01	−0.05 to 0.08	0.723	0.02	−0.04 to 0.09	0.480	40	−0.01	−0.04 to 0.03	0.732
Caudate	CPD	53	−6.1E−04	−0.04 to 0.04	0.974	−0.02	−0.06 to 0.02	0.250	−0.04	−0.13 to 0.04	0.322	1.9E−03	−0.10 to 0.10	0.969	50	−0.02	−0.04 to 0.01	0.171
Hippocampus	CPD	19	0.02	−0.03 to 0.07	0.339	0.03	−0.02 to 0.09	0.246	0.04	−0.03 to 0.11	0.302	−0.05	−0.24 to 0.13	0.575	19	0.03	−0.01 to 0.06	0.177
Pallidum	CPD	25	0.01	−0.02 to 0.05	0.488	−0.01	−0.06 to 0.03	0.553	−0.03	−0.08 to 0.03	0.403	0.05	−0.02 to 0.13	0.155	25	0.01	−0.02 to 0.04	0.485
Putamen	CPD	35	−6.9E−03	−0.04 to 0.02	0.664	2.8E−04	−0.04 to 0.04	0.989	0.01	−0.04 to 0.06	0.636	0.02	−0.05 to 0.10	0.544	34	1.4E−03	−0.03 to 0.03	0.917
Thalamus	CPD	12	0.03	−0.07 to 0.03	0.541	0.04	−0.05 to 0.12	0.390	0.02	−0.13 to 0.17	0.802	0.08	−0.19 to 0.35	0.569	11	0.06	0.00–0.12	0.037
Nucleus accumbens	Alcohol dependence	13	−0.01	−0.12 to 0.01	0.922	−0.06	−0.22 to 0.10	0.446	−0.09	−0.34 to 0.17	0.514	0.26	−0.06 to 0.57	0.134	13	−0.01	−0.12 to 0.10	0.884
Amygdala $P < 5E{-}08$	Alcohol dependence	2	0.09	−0.18 to 0.36	0.522	–	–	–	–	–	–	–	–	–	–	–	–	–
Amygdala $P < 1E{-}05$	Alcohol dependence	39	0.08	1.2E−03 to 0.05	0.046	0.10	−0.01 to 0.22	0.081	0.09	−0.04 to 0.21	0.193	0.01	−0.15 to 0.16	0.916	39	0.08	0.00–0.15	0.059
Caudate	Alcohol dependence	53	0.02	−0.04 to 0.08	0.521	3.2E−03	−0.08 to 0.09	0.942	−6.5E−03	−0.19 to 0.07	0.921	−0.07	−0.22 to 0.09	0.455	53	0.02	−0.04 to 0.07	0.513
Hippocampus	Alcohol dependence	19	0.07	−0.03 to 0.07	0.156	0.09	−0.03 to 0.21	0.158	0.11	−0.24 to 0.47	0.280	0.08	−0.30 to 0.46	0.667	19	0.07	−0.03 to 0.17	0.167
Pallidum	Alcohol dependence	25	0.01	−0.06 to 0.08	0.796	−0.05	−0.15 to 0.05	0.326	−0.04	−0.16 to 0.09	0.560	−0.05	−0.21 to 0.10	0.508	25	0.01	−0.06 to 0.08	0.796
Putamen	Alcohol dependence	36	−0.03	−0.11 to 0.06	0.563	0.02	−0.09 to 0.13	0.700	0.04	−0.07 to 0.16	0.465	0.02	−0.20 to 0.23	0.870	35	−4.2E−03	−0.07 to 0.06	0.900
Thalamus	Alcohol dependence	13	0.03	−0.13 to 0.08	0.748	0.05	−0.11 to 0.21	0.538	0.08	−0.14 to 0.30	0.506	0.40	−0.16 to 0.96	0.180	12	0.04	−0.07 to 0.16	0.458
Nucleus accumbens	DPW	13	0.01	−0.04 to 0.06	0.626	−3.6E−03	−0.04 to 0.03	0.856	−0.01	−0.07 to 0.04	0.616	−0.15	−0.28 to 0.02	0.039	11	0.01	−0.02 to 0.04	0.456
Amygdala $P < 5E{-}08$	DPW	2	0.06	−0.01 to 0.12	0.098	–	–	–	–	–	–	–	–	–	–	–	–	–
Amygdala $P < 1E{-}05$	DPW	40	0.01	−0.01 to 0.03	0.289	0.01	−0.01 to 0.04	0.388	0.01	−0.03 to 0.05	0.520	−0.04	−0.08 to 0.7E−03	0.048	40	0.01	−0.01 to 0.03	0.239
Caudate	DPW	52	2.8E−03	−0.02 to 0.02	0.784	−4.6E−03	−0.02 to 0.02	0.648	9.8E−03	−0.02 to 0.04	0.481	0.02	−0.04 to 0.07	0.535	44	−0.01	−0.02 to 0.01	0.285
Hippocampus	DPW	19	0.01	−0.02 to 0.04	0.472	0.02	−0.01 to 0.04	0.271	0.02	−0.02 to 0.05	0.448	−0.19	−0.29 to 0.09	0.002	18	6.7E−04	−0.02 to 0.02	0.945
Pallidum	DPW	25	−0.01	−0.04 to 0.01	0.304	−0.02	−0.04 to 4.3E−03	0.116	−0.02	−0.05 to 5.0E−03	0.128	−0.03	−0.08 E 0.03	0.326	23	−0.02	−0.03 to 0.00	0.049
Putamen	DPW	34	−5.5E−03	−0.02 to 0.01	0.576	−4.9E−03	−0.02 to 0.01	0.628	−2.3E−03	−0.02 to 0.02	0.839	−0.01	−0.07 to 0.05	0.742	33	−0.01	−0.02 to 0.01	0.200
Thalamus	DPW	13	1.0E−03	−0.04 to 0.04	0.957	−0.01	−0.04 to 0.03	0.701	−0.01	−0.07 to 0.05	0.740	0.18	0.09–0.28	0.002	13	−1.4E−03	−0.03 to 0.02	0.915

SNP, Single nucleotide polymorphism; IVW, inverse-variance weighted regression analysis; GSMR, generalised summary level Mendelian randomisation; CPD, cigarettes smoked per day; DPW, alcoholic drinks per week.
a. MR-Egger reported simulation extrapolation (SIMEX)-corrected values if the IGX^2 statistic (regression dilution) was <0.9, or, if the IGX2 statistic was <0.6 nothing was reported. If the number of SNPs included in the $P < 5E{-}08$ instrument was <10, a lowered SNP inclusion threshold ($P < 1E{-}05$) was also reported.

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Table 3 Mendelian randomisation analysis, with smoking and alcohol use as the exposures and subcortical brain volumes as the outcomes

		SNP	IVW			Weighted median			Weighted mode			MR-Egger[a]			SNP	GSMR		
Exposure	Outcome	*n*	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*β*-value	95% CI	*P*-value	*n*	*β*-value	95% CI	*P*-value
Smoking initiation	Nucleus accumbens	346	−0.04	−0.10 to 0.01	0.114	−0.03	−0.10 to 0.04	0.381	−0.02	−0.13 to 0.09	0.743	–	–	–	339	−0.05	−0.09 to 0.00	0.050
Smoking initiation	Amygdala	346	−0.05	−0.07 to −9.3E−04	0.046	4.0E−04	−0.07 to 0.07	0.991	0.01	−0.11 to 0.09	0.856	–	–	–	340	−0.05	−0.09 to 0.00	0.058
Smoking initiation	Caudate	346	3.33E−03	−0.10 to 0.07	0.919	−0.02	−0.10 to 0.06	0.660	−6.0E−03	−0.14 to 0.13	0.929	–	–	–	335	−0.01	−0.06 to 0.04	0.671
Smoking initiation	Hippocampus	346	−2.20E−03	−0.09 to 0.05	0.939	−0.02	−0.09 to 0.06	0.680	6.51E−04	−0.13 to 0.13	0.992	–	–	–	340	−0.02	−0.07 to 0.03	0.449
Smoking initiation	Pallidum	346	−0.04	−0.09 to 0.02	0.166	−0.02	−0.09 to 0.05	0.502	−0.01	−0.14 to 0.13	0.934	–	–	–	338	−0.03	−0.08 to 0.02	0.206
Smoking initiation	Putamen	346	0.02	−0.09 to 0.08	0.614	−0.01	−0.09 to 0.06	0.728	−0.03	−0.15 to 0.09	0.618	–	–	–	336	0.01	−0.04 to 0.06	0.614
Smoking initiation	Thalamus	345	−0.03	−0.09 to 0.03	0.304	−0.02	−0.09 to 0.06	0.640	−0.01	−0.13 to 0.12	0.900	–	–	–	340	−0.01	−0.06 to 0.04	0.666
Alcohol dependence	Nucleus accumbens	10	−0.06	−0.21 to 0.05	0.301	−0.06	−0.21 to 0.08	0.388	−0.06	−0.26 to 0.13	0.545	−0.32	−0.77 to 0.12	0.189	10	−0.07	−0.20 to 0.06	0.309
Alcohol dependence	Amygdala	10	−0.15	−0.31 to −0.04	0.007	−0.17	−0.31 to −0.04	0.013	−0.17	−0.34 to −0.01	0.074	−0.28	−0.60 to 0.05	0.276	10	−0.15	−0.28 to −0.02	0.023
Alcohol dependence	Caudate	10	0.04	−0.14 to 0.16	0.552	2.1E−03	−0.14 to 0.14	0.977	0.01	−0.17 to 0.18	0.934	0.49	−0.02 to 1.01	0.099	10	0.02	−0.11 to 0.15	0.734
Alcohol dependence	Hippocampus	10	−0.11	−0.26 to −0.01	0.037	−0.11	−0.26 to 0.04	0.156	−0.08	−0.28 to 0.11	0.417	−0.50	−0.87 to −0.13	0.031	10	−0.12	−0.25 to 0.01	0.077
Alcohol dependence	Pallidum	10	−0.02	−0.13 to 0.11	0.756	0.03	−0.13 to 0.18	0.737	0.12	−0.07 to 0.32	0.252	−0.50	−1.08 to 0.08	0.130	10	0.01	−0.12 to 0.14	0.883
Alcohol dependence	Putamen	10	−0.03	−0.16 to 0.08	0.624	−0.01	−0.16 to 0.13	0.874	−0.02	−0.21 to 0.16	0.806	0.13	−0.37 to 0.62	0.630	10	−0.01	−0.14 to 0.12	0.891
Alcohol dependence	Thalamus	10	−0.09	−0.21 to 0.02	0.097	−0.06	−0.21 to 0.09	0.443	−0.03	−0.21 to 0.15	0.774	−0.46	−0.91 to −3.7E−03	0.084	10	−0.01	−0.14 to 0.12	0.891
DPW	Nucleus accumbens	92	−0.13	−0.39 to 0.04	0.122	−0.18	−0.39 to 0.04	0.111	−0.17	−0.38 to 0.05	0.137	−0.18	−0.46 to 0.10	0.200	86	−0.13	−0.26 to 0.01	0.070
DPW	Amygdala	92	−0.11	−0.46 to 0.04	0.147	−0.25	−0.46 to −0.03	0.025	−0.24	−0.48 to −2.1E−04	0.053	−0.17	−0.42 to 0.07	0.174	87	−0.13	−0.27 to 0.00	0.057
DPW	Caudate	92	0.20	−0.06 to 0.39	0.032	0.18	−0.06 to 0.41	0.138	0.09	−0.12 to 0.31	0.401	0.14	−0.16 to 0.45	0.361	85	0.14	0.00–0.28	0.054
DWP	Hippocampus	92	−4.67E−03	−0.34 to 0.14	0.949	−0.14	−0.34 to 0.06	0.170	−0.18	−0.40 to 0.03	0.100	−0.10	−0.33 to 0.14	0.411	87	−0.03	−0.17 to 0.10	0.635
DPW	Pallidum	92	−0.15	−0.52 to 0.03	0.096	−0.29	−0.52 to −0.05	0.017	−0.31	−0.53 to −0.09	0.007	−0.39	−0.68 to −0.10	0.010	87	−0.17	−0.31 to −0.04	0.013
DPW	Putamen	92	−0.01	−0.29 to 0.19	0.896	−0.06	−0.29 to 0.17	0.605	−0.13	−0.36 to 0.10	0.258	−0.12	−0.45 to 0.21	0.482	83	−0.01	−0.15 to 0.13	0.839
DPW	Thalamus	92	−0.13	−0.48 to 0.03	0.104	−0.26	−0.48 to −0.04	0.022	−0.27	−0.50 to −0.04	0.025	−0.21	−0.46 to 0.05	0.118	86	−0.13	−0.27 to 0.00	0.058
$CPD^{Smokers}$	Nucleus accumbens	49	2.28	−8.14 to 12.71	0.668	−1.41	−14.32 to 11.51	0.831	2.75	−7.42 to 12.92	0.598	9.95	−5.16 to 25.07	0.203	45	6.77	−0.07 to 19.43	0.294
$CPD^{Smokers}$	Amygdala	49	8.41	−14.10 to 30.91	0.464	12.86	−14.93 to 40.65	0.365	7.27	−17.02 to 31.56	0.560	5.02	−28.22 to 38.25	0.769	45	5.62	−10.38 to 35.2	0.710
$CPD^{Smokers}$	Caudate	49	−18.59	−60.66 to 23.48	0.386	−23.17	−71.06 to 24.72	0.343	−30.68	−74.45 to 13.09	0.176	−59.16	−119.27 to 0.95	0.060	44	−13.30	−41.1 to 38.09	0.612
$CPD^{Smokers}$	Hippocampus	49	−94.73	−133.57 to −55.89	1.8E−06	−121.36	−170.28 to −72.43	1.2E−06	−108.97	−153.96 to −63.99	1.9E−05	−106.98	−164.19 to −49.77	0.001	45	−46.57	−75.96 to 7.78	0.093
$CPD^{Smokers}$	Pallidum	49	−13.43	−44.75 to 17.89	0.401	−21.67	−51.32 to 7.98	0.152	−23.51	−50.45 to 3.44	0.094	−1.89	−47.95 to 44.18	0.936	45	7.19	−8.43 to 36.07	0.626
$CPD^{Smokers}$	Putamen	49	−68.86	−125.68 to −12.05	0.018	−56.36	−119.91 to 7.18	0.082	−41.29	−90.27 to 7.69	0.105	−7.59	−88.06 to 72.87	0.854	45	−30.52	−64.02 to 31.43	0.334
$CPD^{Smokers}$	Thalamus	49	−1.95	−55.21 to 51.30	0.943	27.72	−40.07 to 95.51	0.423	0.60	−54.88 to 56.07	0.983	−5.23	−83.93 to 73.47	0.897	44	22.31	−13.84 to 89.18	0.513
$CPD^{NeverSmokers}$	Nucleus accumbens	49	−9.50	−17.86 to −1.14	0.026	−11.83	−21.82 to −1.83	0.020	−12.70	−21.59 to −3.82	0.007	−16.20	−28.26 to −4.14	0.011	45	−5.72	−15.86 to 4.43	0.269
$CPD^{NeverSmokers}$	Amygdala	49	−15.66	−34.05 to 2.73	0.095	−20.19	−42.69 to 2.30	0.079	−19.79	−39.25 to −0.33	0.052	−20.93	−48.02 to 6.15	0.136	44	4.83	−18.75 to 28.41	0.688
$CPD^{NeverSmokers}$	Caudate	49	−12.17	−50.53 to 26.19	0.534	−8.08	−52.37 to 36.21	0.721	−18.29	−53.10 to 16.52	0.308	−36.42	−92.27 to 19.42	0.207	43	0.29	−41.27 to 41.86	0.989
$CPD^{NeverSmokers}$	Hippocampus	49	−30.40	−60.75 to −0.04	0.050	−26.96	−66.70 to 12.79	0.184	−24.92	−62.12 to 12.28	0.195	−9.42	−53.78 to 34.94	0.679	45	−21.44	−65.2 to 22.32	0.337
$CPD^{NeverSmokers}$	Pallidum	49	−16.57	−44.82 to 11.69	0.250	−35.33	−61.56 to −9.10	0.008	−23.44	−44.09 to −2.80	0.031	−16.97	−58.70 to 24.76	0.429	43	11.91	−14.08 to 37.9	0.369
$CPD^{NeverSmokers}$	Putamen	49	−71.41	−119.23 to −23.58	0.003	−69.84	−116.59 to −23.08	0.003	−66.83	−108.38 to −25.28	0.003	−76.98	−147.58 to −6.37	0.038	44	−53.17	−101.94 to −4.39	0.033
$CPD^{NeverSmokers}$	Thalamus	49	−45.30	−92.05 to 1.46	0.058	−67.14	−117.07 to −17.20	0.008	−52.49	−92.41 to −12.57	0.013	−65.05	−133.66 to 3.57	0.069	44	−17.06	−70.36 to 36.24	0.530

Note that for the analyses where cigarettes per day (CPD) was the exposure (stratified on smoking status), SNP effects for subcortical brain volume were in units of millimetres cubed, as opposed to constructed beta-coefficients and s.e. for all other analyses, which explains the difference in the size of the causal estimates. SNP, Single nucleotide polymorphism; IVW, inverse-variance weighted regression analysis; GSMR, generalised summary level Mendelian randomisation; CPD, cigarettes smoked per day; DPW, alcoholic drinks per week; $CPD^{Smokers}$, cigarettes smoked per day among ever smokers; $CPD^{NeverSmokers}$, cigarettes smoked per day among never smokers.

a. MR-Egger reported simulation extrapolation (SIMEX) -corrected values if the IGX^2 statistic (regression dilution) was <0.9, or, if if the IGX^2 statistic was <0.6 nothing was reported.

respectively). Similarly, there was weak evidence for an effect of cigarettes per day on amygdala and thalamus volume in never smokers, pointing to horizontal pleiotropy.

There was evidence for a decreasing effect of liability to alcohol dependence on hippocampal volume ($\beta_{IVW} = -0.11$, $P = 0.037$), and strong evidence for a decreasing effect on amygdala volume ($\beta_{IVW} = -0.15$, $P = 0.007$), consistent across weighted median, weighted mode, MR-Egger and GSMR (Supplementary Figs 3 and 4). There was weak evidence for horizontal pleiotropy for alcohol dependence to hippocampal volume (MR-Egger intercept 0.023, $P = 0.080$) and no clear evidence for alcohol dependence to amygdala volume (MR-Egger intercept 0.007, $P = 0.468$). There was no clear evidence for heterogeneity in the SNP effects ($P = 0.950$ and $P = 0.691$, respectively). MR-PRESSO and Steiger filtering did not identify SNP outliers.

There was weak evidence that alcohol dependence decreases thalamus volume ($\beta_{IVW} = -0.09$, $P = 0.097$), corroborated by MR-Egger, but not the other methods. There was no clear evidence for horizontal pleiotropy (MR-Egger intercept 0.021, $P = 0.150$) or heterogeneity ($P = 0.493$). No SNPs were excluded with MR-PRESSO or Steiger filtering.

There was evidence that more alcoholic drinks per week increases caudate volume ($\beta_{IVW} = 0.20$, $P = 0.032$). The effect was consistent with weighted median, but attenuated with weighted mode, MR-Egger and GSMR. There was no clear evidence for horizontal pleiotropy (MR-Egger intercept 0.001, $P = 0.629$), but strong evidence for heterogeneity ($P = 6.4E{-}10$). MR-PRESSO identified five SNP outliers, which did not distort the causal estimate. Steiger filtering identified 26 SNPs that explained more variance in the outcome than the exposure and, after removing these (leaving 66 SNPs), no evidence for causality remained. Taken together, evidence that alcoholic drinks per week causally increases caudate volume is weak.

There was weak evidence that more alcoholic drinks per week decreases pallidum volume ($\beta_{IVW} = -0.15$, $P = 0.096$), consistent and even stronger in size and statistical evidence across weighted median, weighted mode, MR-Egger and GSMR. There was evidence for horizontal pleiotropy (MR-Egger intercept 0.003, $P = 0.049$) and strong evidence for heterogeneity ($P = 2.2E{-}08$). MR-PRESSO identified two SNP outliers, but there was no distortion in the causal estimate after removal. With Steiger filtering, 35 SNPs were excluded (leaving 58 SNPs), but weak evidence for a causal effect remained.

Finally, from alcoholic drinks per week toamygdala and thalamus volumes, there were sizable negative effects. While the main IVW method provided no clear statistical evidence for causal relationships, the effects appeared much stronger in effect size and statistical evidence with the other sensitivity methods (Table 3). There was no horizontal pleiotropy (MR-Egger intercept 0.001, $P = 0.537$ and MR-Egger intercept 0.001, $P = 0.459$, respectively) but there was evidence for heterogeneity ($P = 0.005$ and $P = 0.001$, respectively). There were no SNP outliers with MR-PRESSO and, although Steiger filtering excluded 27 and 25 SNPs, respectively, weak evidence for causality remained.

Discussion

This is the first Mendelian randomisation study to assess bi-directional, causal relationships between volume of subcortical brain regions and substance use behaviours. Our most robust findings indicated that liability to alcohol dependence causally decreases amygdala and hippocampal volume, and that smoking more cigarettes per day causally decreases hippocampal volume.

The evidence that alcohol dependence decreases amygdala and hippocampal volume is in line with findings that subcortical brain regions in individuals with alcohol dependence are smaller and have a lower thickness and surface area than in healthy controls (particularly the amygdala and hippocampus).[9–11,14,36,37] Given Mendelian randomisation's powerful premise and the consistency of our findings across sensitivity analyses, we can make stronger conclusions that this is a result of causal effects. It had previously been hypothesised that alcohol can cause cell death/reduced cell density, resulting in volume loss.[36] For instance, chronic alcohol consumption induces tumour necrosis factor alpha, a cytokine involved in potentiating neuroinflammation, which can cause neuronal death.[38] When number of alcoholic drinks per week was the exposure, we found only weak evidence that more drinks decreases amygdala, pallidum and thalamus volumes, and no clear evidence for hippocampus volume. This discrepancy is likely, in part, because alcohol dependence is the more severe phenotype, reflecting prolonged and heavy exposure of the brain to alcohol. It may also be that downstream effects of alcohol dependence affect the brain, such as poor nutrition, social isolation or psychiatric comorbidity, rather than alcohol itself.

We found strong evidence that smoking more cigarettes (in smokers) decreases hippocampal volume and weak evidence that initiating smoking decreases amygdala volume, implying that smoking can induce structural subcortical brain changes. Although the literature on biological mechanisms responsible for such effects is scarce, animal models have shown that exposure to nicotine can induce apoptosis in hippocampal cells.[39] In contrast to our findings, and to those of observational studies,[4–7] a large ENIGMA study found smoking to be associated with greater thickness and surface area of subcortical regions.[14] This discrepancy might be because the ENIGMA study was observational and influenced by confounders.

There is ongoing discussion whether differences in brain structure between substance users and controls reflect pre-existing differences, or are the result of alterations caused by substance use. Our results mostly point to the latter, with robust evidence for negative effects of liability to alcohol dependence and smoking on some subcortical volumes, without (similarly robust) evidence for causal effects from subcortical volumes to substance use. This is important knowledge, with potentially far-reaching consequences. Volume loss might lead to cognitive deficits and a higher chance of mental illness, since smaller amygdala and hippocampus volumes are implicated in psychiatric disorders.[40] For instance, it is plausible that smoking-related structural brain changes in regions that connect fear response areas (e.g. amygdala) affect trait anxiety, subsequently leading to an anxiety disorder.[41] More research is needed to test pathways from smoking and alcohol use to subcortical brain volume, and subsequently, psychiatric symptoms.

Strengths and limitations

This study has important strengths. We used large genetic data-sets, allowing us to test causal effects with sufficiently powered genetic instruments in both directions. We applied an extensive set of sensitivity methods to assess the robustness of our findings, allowing claims about causality with considerable certainty. It is unlikely that our findings were distorted by variations in explained variance (and thus statistical power) between instruments, given that the most robust evidence for causality was found with genetic instruments of comparably low explained variance. It is also unlikely that (genetic) overlap between smoking and alcohol use has affected our results, given that we uncovered distinctly different patterns of causal effects on (some) subcortical regions for smoking versus alcohol use, and that genetic correlations are low or moderate (alcoholic drinks per week with smoking initiation ($r_g = 0.34$) and with smoking cessation ($r_g = -0.11$) were Bonferroni-corrected significant; alcoholic drinks per week with cigarettes per day ($r_g = 0.07$) was not significant).[19]

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There are, however, limitations to consider. The identified causal relationships may not exhaustively represent causal chains. Although many techniques were used to correct for horizontal pleiotropy, vertical pleiotropy (some other factor mediating the exposure) cannot be ruled out. In addition, because we used separate samples to obtain exposure and outcome estimates, we were unable to ascertain if individuals in the outcome sample were affected by said exposure (apart from cigarettes per day, for which we stratified on smoking status). Therefore, we should interpret all causal effects as an effect of the liability to that exposure. It is also important to note that causal effects found with Mendelian randomisation should be interpreted as lifetime exposures.[42] Although we included adult participants and the GWAS were corrected for age (Supplementary Table 2), our results do not provide information on temporal patterns. In addition, it should be noted that our findings result from samples of European ancestry, which limits the interpretability in other populations. This is a common limitation in the field of genetics, as up to 2017, ~88% of GWAS studies were based on populations solely of European descent.[43] Follow-up research with a higher geographic coverage would be highly recommended to investigate if our findings are mirrored in populations of non-European descent. Finally, although Mendelian randomisation can provide powerful causal inference, there may be bias from 'genetic nurturing' (the genotype of parents directly affecting offspring phenotypes even if the responsible variants are not transmitted), 'assortative mating' (spouses showing higher phenotypic and genotypic similarity than expected by chance) and geographic clustering. These phenomena reintroduce bias from confounding. When such data become available, future Mendelian randomisation studies should use genetic estimates from large-scale within-family GWAS, as these can correct for more fine-grained (geographical/family) clustering.[44]

In conclusion, we report robust evidence that liability to alcohol dependence causally affects the brain, decreasing subcortical volume (at least pertaining to the hippocampus and amygdala). For smoking there was strong evidence that it causally decreases hippocampal volume, and more tentatively, amygdala volume. These findings provide additional proof that smoking and alcohol use can be detrimental to the brain, and it may implicate structural changes as a pathway connecting substance use to the development of psychiatric disorders. We feel that, combined with accumulating evidence from other types of research, this justifies more recognition in public health efforts and clinical practice.

Emma Logtenberg, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Martin F. Overbeek**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Joëlle A. Pasman**, Behavioural Science Institute, Radboud University Nijmegen, The Netherlands; **Abdel Abdellaoui**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Maartje Luijten**, Behavioural Science Institute, Radboud University Nijmegen, The Netherlands; **Ruth J. van Holst**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Jacqueline M. Vink**, Behavioural Science Institute, Radboud University Nijmegen, The Netherlands; **Damiaan Denys**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Sarah E. Medland**, Psychiatric Genetics Group, QIMR Berghofer Medical Research Institute, Australia; **Karin J. H. Verweij**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands; **Jorien L. Treur**, Department of Psychiatry, Amsterdam UMC, University of Amsterdam, The Netherlands

Correspondence: Jorien L. Treur. Email: j.l.treur@amsterdamumc.nl

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Supplementary material

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Data availability

The data that support the findings of this study include summary-level data of published GWAS, all of which are publicly available or can be requested from the corresponding author, J.L.T. Some of the analyses were based on data from UK Biobank, which cannot be provided by the authors but can be requested from UK Biobank directly.

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Author contributions

J.L.T. and K.J.H.V. conceived and designed the study. E.L. and M.F.O. conducted the data analyses, with help from J.L.T., A.A. and J.A.P.. J.L.T. wrote the manuscript, with significant contributions from E.L. and M.F.O.. M.L., R.J.V.-H., J.M.V., D.D. and S.E.M. contributed specific expert insights in neuro-imaging and clinical implications. All authors critically reviewed the report, proposed revisions and approved the final manuscript for publication.

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Declaration of interest

None.

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