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Mapping cortical and subcortical asymmetries in substance dependence: Findings from the ENIGMA Addiction Working Group

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Abstract

Brain asymmetry reflects left-right hemispheric differentiation, which is a quantitative brain phenotype that develops with age and can vary with psychiatric diagnoses. Previous studies have shown that substance dependence is associated with altered brain structure and function. However, it is unknown whether structural brain asymmetries are different in individuals with substance dependence compared to nondependent participants. Here, a mega-analysis was performed using a collection of 22 structural brain MRI datasets from the ENIGMA Addiction Working Group. Structural asymmetries of cortical and subcortical regions were compared between individuals who were dependent on alcohol, nicotine, cocaine, methamphetamine, or cannabis ($n = 1,796$) and nondependent participants ($n = 996$). Substance-general and substance-specific effects on structural asymmetry were examined using separate models. We found that substance dependence was significantly associated with differences in volume asymmetry of the nucleus accumbens (NAcc; less rightward; Cohen's $d = 0.15$). This effect was driven by differences from controls in individuals with alcohol dependence (less rightward; Cohen's $d = 0.10$) and nicotine dependence (less rightward; Cohen's $d = 0.11$). These findings suggest that disrupted structural asymmetry in the NAcc may be a characteristic of substance dependence.

Introduction

Dependence on alcohol and other substances is characterized by a variety of symptoms, including impaired control, social disruption, risky behaviors, and increased substance tolerance and withdrawal symptoms. In the United States alone, an estimated 164.8 million people aged 12 or older in 2018 used alcohol, tobacco or illicit substances in the month before survey, and about 20.3 million people had [substance use problems](#) in the prior year (1). Substance dependence imposes a substantial disease burden on individuals and society (2). Consequently, understanding the neurobiological underpinnings of substance dependence has been a long-standing topic in neuroscientific studies.

A substantial body of evidence now supports the view that there are structural differences between the brains of individuals diagnosed with substance dependence and nondependent participants (3-5). For example, significantly smaller volume in right dorsolateral-prefrontal cortex, right anterior insula, and right nucleus accumbens (NAcc), as well as left amygdala, was observed in abstinent chronic alcoholic men ($N = 21$) [as compared to healthy control men \(\$N = 21\$ \)](#) (4). Smaller thickness of left medial orbitofrontal cortex was observed in smokers ($N = 22$) compared to never-smokers ($N = 21$) (3) and smaller frontal volumes have also been reported in individuals who used cocaine and amphetamine-type stimulants (6). A recent mega-analysis from the Addiction working group of the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium demonstrated smaller subcortical volumes in the bilateral amygdala and hippocampus but only in the right NAcc of individuals who were primarily dependent on one of five substances (i.e., alcohol, nicotine, cocaine, methamphetamine or cannabis) ($n = 2,140$) compared with nondependent participants ($n = 1,100$) (5). Although structural differences associated with substance dependence are

typically reported for each hemisphere separately and both bilateral and unilateral effects have been observed, very few studies have examined lateralization effects *per se*.

Brain lateralization is a quantitative brain phenotype that varies as a function of age, sex, and psychiatric diagnosis (7, 8). It reflects left-right hemispheric differentiation and has replicable heritability, making it useful for studies on health or disease (8). In a large sample of healthy adults ($n = 17,141$), Kong and colleagues found a global pattern of hemispheric asymmetries in cortical thickness where posterior brain regions were more right-lateralized and anterior brain regions were more left-lateralized (8). Also, alterations in cortical and subcortical asymmetry were observed in patients with psychiatric disorders, such as autism ($N = 3,583$) and obsessive-compulsive disorder ($N = 3,431$) (9, 10).

Atypical functional lateralization has been observed in prior studies of substance dependence (11, 12). For instance, a recent meta-analysis reported that substance dependent individuals exhibit lower right anterior activation during the Go/No-Go studies (15 studies with 409 participants and 218 peaks) and the Stop Signal tasks (12 studies with 274 participants and 156 peaks) compared to nondependent participants (11). Findings from previous functional MRI studies suggest that substance use affects left and right brain functions differently and the degree of lateralization of brain activation may be a potential risk factor for addiction (11, 12). A separate study with healthy participants ($N=106$) demonstrated hemispheric lateralization of resting-state functional connectivity of the ventral striatum, a structure widely implicated in the etiological processes of addiction (13). However, to date, no study with a large sample has explored structural asymmetries in substance dependent individuals and compared them to nondependent controls.

Here, we used neuroimaging data from the ENIGMA consortium's Addiction working group to compare structural asymmetries between substance dependent and nondependent participants following an analysis approach developed in previous studies of brain asymmetries (10). Brain asymmetry was calculated as $(L-R)/(L+R)$ separately for subcortical volume, cortical thickness and surface area and compared between substance dependent and nondependent individuals. The large-scale analysis presented here can overcome the low statistical power and bias issues in smaller individual studies (14, 15). Unilateral and bilateral patterns of structural differences associated with substance dependence have been reported previously (5). Structural and functional differences in right ventral striatum in substance dependence as compared to nondependent participants have been reported (5, 16, 17). We therefore were particularly interested in determining if these seemingly lateralized effects translated into measurable asymmetries in brain structure and if they were specific to certain substances or addiction in general.

Methods

Behavioral phenotyping

Data were gathered from 22 laboratories on 3,046 individuals, of whom 1,932 were diagnosed with current dependence on at least one of the five substances of interest: alcohol, nicotine, cocaine, methamphetamine, and cannabis. The data used in the present study were a subset from the previous study by ENIGMA consortium's Addiction Working Group (5). The diagnostic instruments used to assess substance dependence [as defined by the DSM-IV](#) are noted in Table S1 in [the Supplementary Results section](#). Individuals were excluded if they had a lifetime history of neurological diseases, a current DSM-IV axis I diagnosis other than depressive and anxiety disorders, or any contraindication for MRI. Nondependent participants

may have used psychoactive substances recreationally but did not meet DSM-IV criteria for a substance dependence. After quality control steps described below, 2,792 participants including 1,792 dependent participants were included in the present analysis. Summary demographic statistics by group (sex distribution and mean age) for these participants are provided in Table 1. Site-specific summaries are provided in Table S1 in [the Supplementary Results section](#).

Preparation of structural MRI data

The volumes of 7 bilateral subcortical regions, and thicknesses and surface areas of 34 bilateral cortical regions from both hemispheres were extracted from structural T1-weighted MRI brain scans using FreeSurfer (version 5.3) (18). A standardized protocol of quality control procedures was performed at each site (<http://enigma.ini.usc.edu/protocols/imaging-protocols/>), which includes detection of outliers and visual inspection of all data in a series of standard planes. An additional visual inspection was performed at the University of Vermont on a randomly selected subsample of participants to ensure uniformity of quality control across sites. Scanner and acquisition details at each site are provided in Table S1 in the online supplement.

Brain asymmetry index

Brain asymmetry index (AI) was calculated with left and right hemisphere data using the following formula: $AI = (L-R)/(L+R)$ separately for subcortical volume, cortical thickness and surface area. Positive and negative AI values denote leftward and rightward asymmetry, respectively. Individuals were excluded from further analysis if they 1) had more than four missing values (i.e., values marked as outliers in the quality control procedure) for the subcortical AIs, or 2) had more than eight missing values for either cortical thickness or surface area AIs. For each AI measure, values that were beyond three standard deviations of the grand

mean of the whole sample were deemed as outliers and were clipped to the three standard deviation value.

Linear mixed-effects models

For each AI of subcortical volume, cortical thickness and surface area, linear mixed-effects (LME) models were fitted to examine substance-general and substance-specific effects on AIs using Model 1 and Model 2 respectively. In Model 1, the substance-general effects on AIs were examined across all individuals who were dependent on any of the five substances relative to nondependent controls. For each AI, an LME model with substance dependence (nondependent or dependent), sex (male or female) and age as fixed effects and testing site as a random effect was fitted. Model 2 examined substance-specific effects on AIs with individuals dummy coded as a member of one of six groups: “nondependent” or dependent on “alcohol,” “nicotine,” “cocaine,” “methamphetamine,” or “cannabis”. In Model 2, the five dummy variables for substances (i.e., the nondependent group was treated as reference), sex (male or female) and age were entered as fixed effects with site as a random effect. Individuals who were dependent on more than one substance were excluded in Model 2. To determine if the effects observed in Model 2 were driven by a specific group, we performed post-hoc comparisons among subgroups for the significant region (i.e., NAcc) identified in Model 2. The FDR-correction was applied to the 15 subgroup contrasts.

The nondependent participants only were included in the already published ENIGMA study that reported the global pattern of cortical lateralization in over 17,000 healthy participants (8). In an additional analysis here, we were interested in reproducing this effect in the nondependent participants included in the current study (n = 996) and examining if this global pattern of cortical lateralization was different in dependent participants (n = 1,729). Therefore, we examined cortical asymmetries of the cortical thickness and surface area in

dependent and nondependent participants separately using LME models, in which hemisphere (left vs. right), sex (male vs. female), age and intracranial volume were entered as fixed effects and site was entered as a random effect. Intracranial volume was included in the analysis to be consistent with the previous study that examined the global pattern of cortical lateralization (8). The resulting Cohen's d values were compared to those published by Kong and colleagues using Pearson correlation.

The LME models were fitted using the “nlme” package (19) in Rstudio (version 1.0.153) and R (version 3.6.2). For the LME model of each region, participants were omitted if any of the variables were missing. To address multiple comparisons, FDR correction (20) was separately applied to p values of subcortical volume (i.e., 7 regions), cortical surface area (i.e., 35 regions including one hemispheric total surface area) and cortical thickness (i.e., 35 regions including one hemispheric total cortical thickness) for Model 1. A same correction strategy was applied for each predictor of interests (i.e., the substance-using group) respectively in Model 2. The main effects of substance use were deemed significant when FDR-corrected $p < .05$, and the corresponding raw p value was reported.

Cohen's d effect sizes and 95% confidence intervals were calculated for the main effect of substance use, with detailed implementation shown in the Supplementary Methods section. Positive Cohen's d effect sizes indicated greater regional AIs in dependent participants compared to nondependent participants, while negative Cohen's d effect sizes indicated lower regional AIs. Greater regional AIs can be interpreted as increased leftward or decreased rightward asymmetry and lower AIs can be interpreted as decreased leftward or increased rightward asymmetry. To determine the direction of the differences between groups, for the regions that showed significant AI differences, estimated marginal means of AI and brain measure (i.e., volume, cortical thickness, or surface area) corresponding to the predictors of

interest (i.e., substance dependence diagnosis) were calculated from the LME models. The *ggseg* package (21) in R was used to visualize the cerebral cortical results, in which Cohen's *d* effect sizes were mapped onto the left cortical and subcortical structures. [The code to implement the analyses included in the present study is available on GitHub \(\[https://github.com/zh1peng/paper_code\]\(https://github.com/zh1peng/paper_code\)\).](#)

To quantify evidence strength for the alternative hypothesis, we complemented the analyses above with a Bayesian approach, reporting the Bayes Factor that quantifies the strength of the evidence in favor of the null or alternative hypothesis (22). For example, the Bayes factor BF_{10}^1 of 5 means that the data are 5 times more likely under H_1 than under H_0 , whereas a Bayes factor BF_{10} of 0.2 can be interpreted as the data are 5 times (i.e., $1/0.2$) more likely under H_0 than under H_1 (22). In the present study, evidence that the data were more likely under the alternative model compared to the null model (i.e., BF_{10}) was calculated using the Bayesian Information Criterion approximation (23), with null models constructed by removing the main effect of interest (e.g., substance use) from the LME models. Per convention, we interpreted Bayes factor (BF_{10}) of < 3 as anecdotal evidence for H_1 , 3-10 as moderate evidence for H_1 and 10-30 as strong evidence for H_1 , 30-100 as very strong evidence for H_1 , and >100 as extreme evidence for H_1 (22).

Past-30-day use

LME models were used to determine whether past-30-day alcohol use was related to the volume/thickness of regions-of-interest identified by Model 1 or 2. [The AIs of NAcc, fusiform and postcentral gyrus surface area were each correlated with past-30-day](#)

¹The 10 in BF_{10} means the level of support for H_1 over H_0 , by contrast with BF_{01} which means support for H_0 over H_1 .

alcohol use using LME models in which amount of the past-30-day alcohol use, age and sex were included as fixed effects and site was entered as a random effect. The AI of NAcc was correlated with past-30-day nicotine use using an LME model in which amount of past-30-day nicotine use, age and sex were included as fixed effects and site was entered as a random effect. The past-30-day use of alcohol or nicotine was based on the self-reported number of individual alcoholic drinks or cigarettes consumed in the past 30 days. Cohen's *d* effect sizes and 95% confidence intervals were calculated for the main effect of the past-30-day substance use, with detailed implementation shown in the Supplementary Methods section. The past-30-day use of alcohol or nicotine was based on the self-reported number of individual alcoholic drinks or cigarettes consumed in the past 30 days. As past-30-day alcohol and nicotine use was only measured in a subset of the studies, the analysis was only performed on 34.7% and 34.2% of dependent participants, respectively.

Support vector machine classification

To explore if there exist patterns of regional asymmetries that could potentially serve as substance dependence predictors, a support vector machine (SVM) with radial basis function kernel classification was implemented using Sklearn (24). To mitigate the effects of site, sex, age and case-only studies, five case-only data sets were excluded, and brain asymmetry was residualized against these variables (i.e., site, sex and age) prior to classification. A 10-fold cross-validation and area under the receiver operating characteristic curve (AUC) were employed to evaluate the performance of the classification. For each fold, data were stratified into testing set (i.e., 10% of the data) and training set (i.e., 90% of the data). The performance of the classification was measured by the AUC on the testing set and the averaged AUC across

folds was reported. Within each fold, parameter tuning was performed in the training set with 15 values in log-space from 0.001 to 100 respectively for C and γ (i.e., $15 \times 15 = 225$ pairs of C and γ in total). Another 10-fold cross-validation (i.e., nested cross-validation) was employed to find the optimal combination of C and γ that maximized the averaged AUC in the training set. The same machine-learning procedure was applied to classify all dependent versus nondependent participants as well as substance-specific dependent versus nondependent participants.

Results

The patterns of leftward and rightward asymmetries for cortical thickness and surface area in both the nondependent controls and dependent participants were similar when compared to the observed patterns of lateralization in the previous findings from over 17,000 healthy participants (see Figure S1 and Table S10). As shown in Figure 1, Model 1 indicated that participants with substance dependence had less rightward asymmetry in the NAcc ($p < 0.001$, Cohen's $d = 0.155$, $BF_{10} = 34.00$) compared to nondependent participants. This result remained significant after an FDR correction for all the subcortical regions and even when corrected for all subcortical and cortical regions combined (i.e., 77 regions) using the Bonferroni method. All other regional AIs were not significantly different between groups, as shown in Table S2.

In Model 2, less rightward asymmetry of the NAcc was observed in the alcohol and nicotine dependent groups (alcohol: $p = 0.007$, Cohen's $d = 0.104$, $BF_{10} = 0.73$; nicotine: $p = 0.006$, Cohen's $d = 0.110$, $BF_{10} = 0.75$). In addition, alcohol dependent participants also showed more leftward asymmetry of postcentral surface area ($p = 0.002$, Cohen's $d = 0.122$, $BF_{10} = 2.71$) but less leftward asymmetry of the fusiform surface area ($p < 0.001$, Cohen's $d =$

-0.127, $BF_{10} = 1.31$) compared with that of nondependent controls. Moreover, cannabis dependent participants demonstrated lower leftward asymmetry of the putamen ($p = 0.004$, Cohen's $d = -0.156$, $BF_{10} = 0.73$) compared to the nondependent controls. The results of LME models for other regions are shown in Tables S3-S7. [Additional plots with residualized data are shown in Figure S8.](#) The results of the post-hoc comparisons showed that only alcohol and nicotine dependent groups had marginally greater NAcc asymmetry as compared to nondependent group with the FDR-correction applied for the 15 contrasts. The results of the post-hoc comparisons are shown in the Table S11.

In the exploratory analysis, none of the regions that showed a significant effect in Model 1 or Model 2 showed association with the past-30-day alcohol or nicotine use (see Tables S8 and S9). In the SVM classification analysis, regional AIs did not successfully classify general substance dependence or specific-substance dependent status with the maximum mean AUC being 0.53. The performance of the SVM classifiers is shown in Figures S2-S7.

Discussion

In this study, a large sample of structural MRI ($N = 2,792$) from the ENIGMA Addiction working group was used to test for associations between structural lateralization in the brain and substance dependence. We found that nondependent and dependent participants shared similar structural lateralization patterns across most regions except for the reduced rightward asymmetry of NAcc in dependent participants. This alteration was observed only in the alcohol- and nicotine-dependent participants in the substance-specific analysis. [However, the NAcc asymmetry was](#) not correlated with the past-30-day alcohol or nicotine use in the alcohol and nicotine dependent participants. In addition, individuals with alcohol dependence had greater leftward asymmetry of postcentral surface area but lower leftward asymmetry of fusiform

surface area, and individuals with cannabis dependence showed less leftward asymmetry of the putamen compared to that of nondependent participants. The SVM classifier with a non-linear kernel could not classify the substance dependence groups based on these structural asymmetry features.

The NAcc showed different lateralization in substance dependent participants compared to nondependent participants. The NAcc is a key component in the reward circuit that is closely related to reinforcement learning and addiction (25). Theoretical models for the etiology of addiction propose that substance intake promotes dopamine release in a more prolonged and unregulated way compared to natural reward. This overstimulation of dopaminergic neurons in the NAcc leads to changes in synaptic plasticity via long-term potentiation processes during repeated exposure to substances (26, 27). A hemispheric difference in the dopaminergic system could contribute to the vulnerability of right NAcc to the overstimulation of repeated substance exposure. For instance, a rightward lateralization of striatal dopamine D2 receptor binding has been reported in prior studies (28, 29). Interestingly, right-lateralized abnormalities in ventral striatal activity has also been observed in substance dependent participants (16, 17). For instance, detoxified alcoholics showed heightened right ventral striatum activity in response to alcohol-associated cues compared to healthy controls (17). A separate study showed alcohol-dependent participants had hypoactivation in the right ventral striatum during reward anticipation in the monetary incentive delay task (16).

In the absence of longitudinal data, the possibility remains that the altered NAcc asymmetry may reflect a predisposition to substance dependence. Indeed, in previous studies, right-lateralized alterations in regions involved in reward processing have been shown to be risk factors for substance dependence (30-32). For instance, more volume reduction was observed in right relative to left orbital frontal cortex in offspring who are at high-risk of alcohol

dependence (30). In a separate positron emission tomography study, reduced dopamine receptor availability in the right ventral striatum was associated with impulsive choices during delay discounting tasks in both alcoholics and social drinkers (31). Therefore, it is possible that abnormalities in NAcc lateralization may disrupt dopamine function, which may contribute as a vulnerability factor for substance dependence. Further research with longitudinal designs is needed to confirm these temporal or causal relations.

Our findings of a decreased rightward asymmetry of the NAcc in substance dependence is consistent with our previous large-scale study showing that right, but not left, NAcc had smaller volume in dependent participants compared to nondependent controls (5). However, it is worth noting that other regions that showed group differences only in left or right hemisphere (in Mackey et al., 2019) did not show significant differences in structural asymmetries in the present study. This underlines the value of a separate examination of brain asymmetries as significant brain asymmetry effects do not necessarily follow from unilateral group differences. Interestingly, despite their relatively smaller sample sizes, both substance dependent ($n = 1,796$) and nondependent ($n = 996$) groups showed similar cortical lateralization patterns to those reported in the previous lateralization study performed in ENIGMA consortium with over 17,000 participants (8). This suggests that brain asymmetries are robust and replicable measures of brain organization across studies. In addition, power analysis with Bayesian Factors indicated that the present data provide strong evidence for there being group differences in NAcc asymmetry ($BF_{10} = 34$). However, Bayesian Factors for the other observed effects of substance dependence on cortical and subcortical structural measures were small (e.g., $BF_{10}=2.73$ for postcentral surface area in Model 2), which underscores the relatively specific NAcc effect.

Most brain regions (76 out of 77 regions examined in Model 1) did not show group differences in structural asymmetry in this study; and the effect size for the group difference in NAcc asymmetry was small (Cohen's $d = 0.155$). This would explain why structural asymmetries could not predict substance dependence in the classification analysis. Although the effect sizes were comparable to those found in previous large-scale studies on brain asymmetries (9, 10), structural asymmetries alone are unlikely to be a strong predictor for substance dependence. In contrast, volumetric measures served as reliable predictors for substance dependence (5), and lateralization of brain activation during tasks has been associated with substance dependence (11, 12). Future studies are needed to determine if brain asymmetries, perhaps measured across multiple imaging modalities, can provide adequate information to improve classification and prediction performance.

The depressive and anxiety disorders are common psychiatric comorbidities among individuals with substance dependence (33), therefore they were not explicitly excluded in the present study. That said, it is possible that the effects observed in NAcc could be driven by the comorbidity of substance dependence and depressive or anxiety disorders. However, we did not have sufficient numbers of participants with the necessary behavioral data to tease apart these possible comorbidity effects on brain asymmetries in the present analysis. In addition, we found that the differences in asymmetry of the NAcc between dependent and nondependent participants were largely driven by individuals with alcohol and nicotine dependence. However, it is possible that the relatively smaller sample sizes in the other substance group might interfere with the detection of subtle effects. These limitations should be borne in mind when interpreting the current results and could be investigated in future studies.

Conclusion

In summary, we performed a large-scale analysis of structural asymmetries in substance dependent and nondependent participants. We found that structural asymmetry of the NAcc was significantly different in substance dependence compared to nondependent controls. These findings suggest that substance dependence may be characterized as disrupted structural asymmetry in NAcc.

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Conflict of Interest

RS has served on the scientific advisory board of Embera Neuro-therapeutics. DJS has received research grants and/or consultancy honoraria from Lundbeck and Sun. MY has received funding from several law firms in relation to expert witness reports. NJ and PT are MPI of a research grant from Biogen, Inc for work unrelated to the contents of this manuscript. All other authors have no financial disclosures.

Authors Contribution

HG, SM and ZC conceived the study and designed the research question. ZC performed the analysis and wrote the original manuscript. HG and SM supervised the study. Other authors contributed to data curation, writing-review and editing manuscript. All authors critically reviewed content and approved final version for publication.

Data availability statement

The data that support the findings of this study are available from the ENIGMA Addiction Working Group upon reasonable request.

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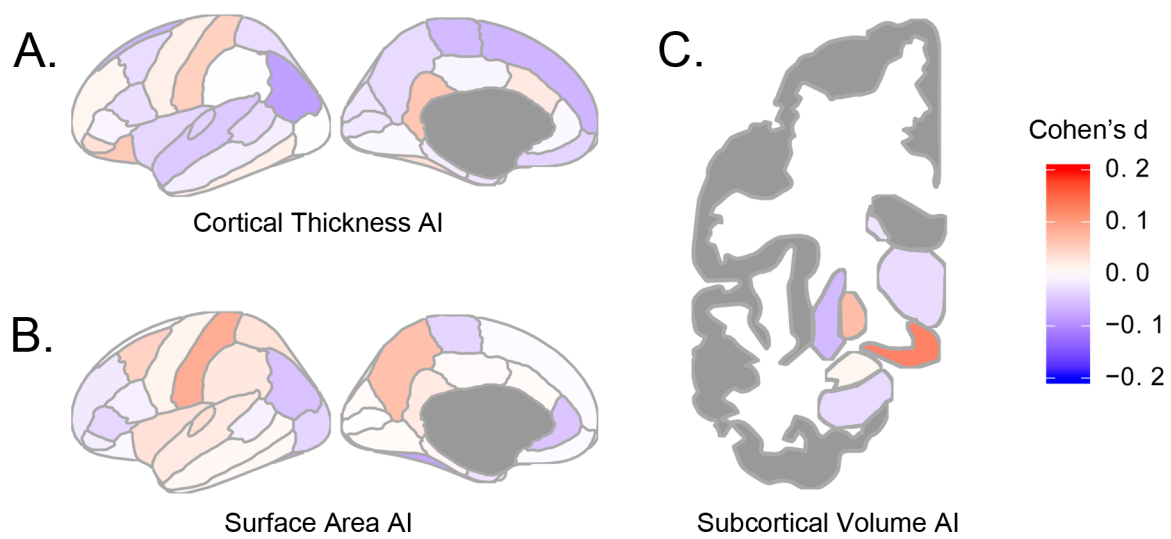
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Tables

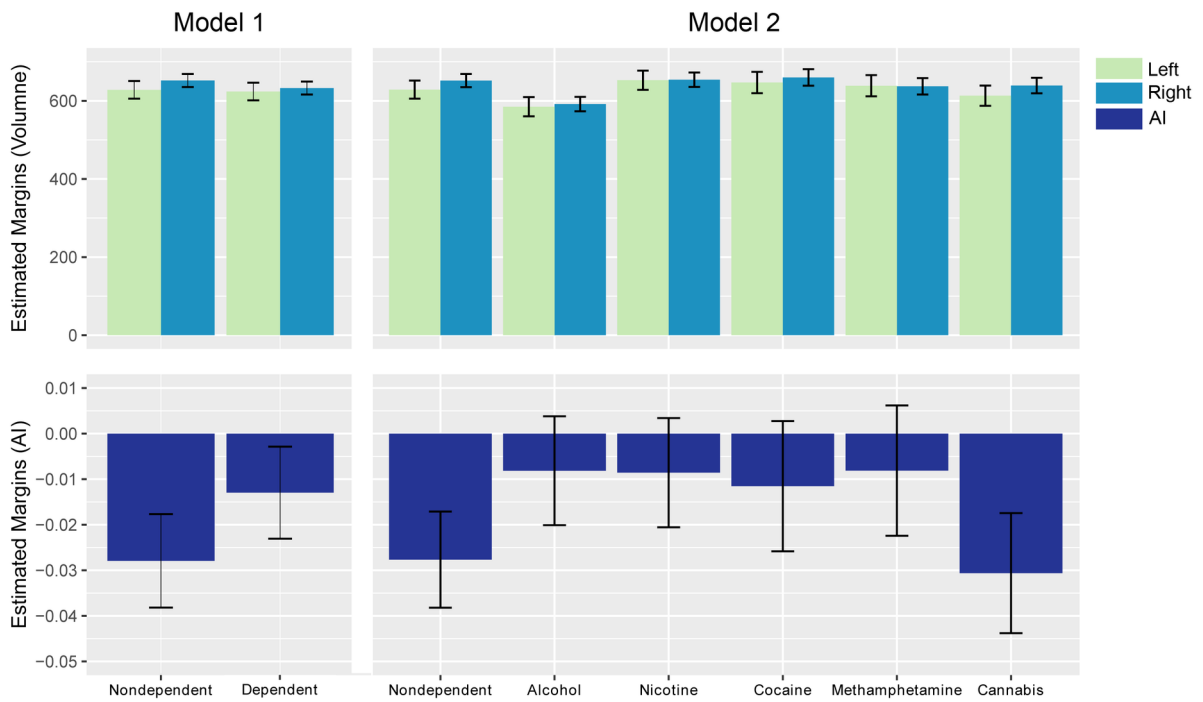
Table 1. Sex distribution and mean age of case and control participants. The chi-squared test and Mann-Whitney-Wilcoxon test were used to examine case-control differences in sex distribution and age separately.

Group or Dependence Subgroup		Total N		Age (years)		
		N	Female	%	Mean	SD
All Groups						
	Dependent	1796	616*	34.30	32.95*	10.56
	Nondependent	996	402	40.36	29.4	9.79
Alcohol						
	Dependent	761	243	31.93	34.11*	10.75
	Nondependent	277	99	35.74	30.70	9.71
Nicotine						
	Dependent	541	232*	42.88	31.19*	9.92
	Nondependent	284	153	53.87	26.21	8.01
Cocaine						
	Dependent	191	47*	24.61	40.65*	7.60
	Nondependent	141	50	35.46	36.91	10.33
Methamphetamine						
	Dependent	116	37	31.90	30.31	8.21
	Nondependent	141	59	41.84	30.48	8.65
Cannabis						
	Dependent	177	51	28.81	26.78	10.06
	Nondependent	153	41	26.80	25.05	8.68

* p<.05



ADB_13010_Figure 1.tif



ADB_13010_Figure 2.tif