Mega-Analysis of Grey Matter Volume in Substance Dependence: General and Substance-Specific Regional Effects

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Abstract

Objective: Although lower brain volume has been routinely observed in individuals with substance dependence compared to non-dependent controls, the brain regions exhibiting lower volume have not been consistent across studies. In addition, it is not clear whether a common set of regions are involved in substance dependence regardless of the substance used or whether some brain volume effects are substance-specific. Resolution of these issues may contribute to the identification of clinically relevant imaging biomarkers.

Method: Brain structure was examined in a mega-analysis of previously published data pooled from 23 labs, including 3,240 individuals of whom 2,140 had a substance dependence on one of five substances: alcohol, nicotine, cocaine, methamphetamine, or cannabis. Subcortical volume and cortical thickness in regions defined by Freesurfer were compared with non-dependent controls when all sampled substance categories were combined, and separately, while controlling for age, sex, imaging site and total intracranial volume. Due to extensive associations with alcohol dependence, a secondary contrast was also performed for dependence on all substances except alcohol. An optimized split half strategy was used to assess the reliability of the findings.

Results: Lower volume/thickness in individuals with substance dependence was observed in many brain regions. The greatest effects were associated with alcohol use disorder. A set of affected regions related to dependence in general regardless of the substance included the insula and the medial orbitofrontal cortex. Further, a support vector machine multivariate classification of regional brain volumes successfully classified individuals with a substance dependence on alcohol or nicotine relative to non-dependent controls.

Conclusions: The results indicate that dependence on a range of different substances shares a common neural substrate and that differential patterns of regional volume could serve as useful biomarkers of dependence on alcohol and nicotine.

Introduction

The social and economic costs associated with problematic use of drugs and alcohol place an enormous burden on the individual and society (1-5). In the United States alone, the National Institute on Drug Abuse estimates that the costs associated with problematic substance use --including medical care, law enforcement and lost productivity-- exceeds $700 billion per year (6). Substance dependence is characterized by a loss of control over drug and alcohol taking behavior, which contributes to high relapse rates (7-10). The therapeutic landscape would be radically altered by the identification of a set of biomarkers that could be used to estimate risk at various stages of the disorder, e.g. the risk of transition from occasional to problematic patterns of use or risk of relapse after treatment, and to prescribe the most appropriate treatment options based on the specific functional vulnerabilities of the individual patient (11, 12).

It remains to be determined whether regional differences in brain volume measured by MRI can provide clinically useful biomarkers of substance dependence. Although brain volumetric studies have routinely observed lower grey matter volume in individuals with substance dependence compared to controls who do not have a substance dependence, the brain regions associated with a substance dependence on a specific substance have not been consistent across studies (13-15). Since volumetric studies have tended to focus on one substance at a time, it is also not clear from this literature whether a shared set of brain areas will exhibit altered volume in all individuals with substance dependence regardless of the substance used. Human twin studies suggest that genetic vulnerability to substance dependence is accounted for principally by a shared set of variations regardless of the substance used with proportionately smaller substance specific effects (16). On the basis of preclinical research and data from other imaging modalities, several candidate brain regions have been proposed to play a central role in substance dependence, including the striatum, the insula and parts of the frontal cortex (reviewed in (17-19)).

The authors of this study joined together to form an international working group within the framework of the ENIGMA Project (20, 21) to overcome issues related to low statistical power in individual neuroimaging studies. This first project of the Addiction working group has pooled data from 23 labs in 14 countries and represents the largest study of brain volumetric data in substance dependence research to date. The objective was to identify general and substance-specific associations between dependence and regional brain volume. The large sample size facilitated the adoption of a rigorous cross-validation method to address the widespread failure to replicate neuroimaging results that has been noted in several recent influential reports (22, 23). In addition, a support vector machine classifier was used to explore patterns of regional brain volume that could potentially serve as disease biomarkers.

Methods

Behavioral Phenotyping

All procedures were performed in accordance with the Declaration of Helsinki. Datasets from the working group were selected that assessed individuals for substance dependence on one of five substances, i.e. alcohol, nicotine, cocaine, methamphetamine and cannabis. A variety of diagnostic instruments was used to assess substance dependence (see Supplemental Table 1). Case/control data were gathered from 23 labs on 3,240 individuals of whom 2,140 were diagnosed with current

dependence on at least one of the five substances of interest: alcohol, nicotine, cocaine, methamphetamine, or cannabis. Subjects were excluded if they had a lifetime history of neurological disease, a current Axis I diagnosis **based on the DSM-IV** except depressive and anxiety related disorders, or endorsed any contraindication for MRI. Control subjects may have used addictive substances recreationally but were not diagnosed as dependent. Summary demographic statistics on participants whose data passed the quality control steps described below are provided in Table 1. Site specific summaries are provided in Supplemental Table 1.

Preparation of Structural MRI Data

Structural T1-weighted MRI brain scans were acquired from all participants. Scanner and acquisition details at each site are provided in Supplemental Table 1. Data were prepared in Freesurfer (version 5.3), a fully automated MRI processing pipeline that identifies 7 bilateral subcortical and 34 bilateral cortical regions-of-interest (24, 25). A majority of the datasets were prepared using CBRAIN, a network of high-performance computing facilities in Canada (26). The volume of subcortical regions-of-interest and mean cortical thickness of cortical regions-of-interest served as the dependent measure in all analyses. The use of Freesurfer in multi-site analyses has been validated in previous ENIGMA publications (27-30) that established a standardized protocol of quality control procedures performed at each site (http://enigma.ini.usc.edu/protocols/imaging-protocols/). This includes detection of outliers and visual inspection of all data in a series of standard planes (see Supplemental Methods for more **details)**. An additional level of visual inspection was performed centrally at the University of Vermont on a randomly selected sub-sample of participants to ensure uniformity of quality control across sites.

Linear Mixed Effects Models with Cross-Validation

Differences in region-of-interest thickness/volume between dependent participants and non-dependent controls were assessed in each region-of-interest with two linear mixed effects models using SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp). The linear mixed effects model effectively accounts for site effects including sites that did not collect non-dependent control data (31)**. In Model 1**, substance dependent individuals were treated as one group regardless of the substance used, i.e. individuals dependent on alcohol, nicotine, cocaine, methamphetamine or cannabis were coded as "dependent" and controls as "non-dependent". Individuals in Model 1 were allowed to be dependent on more than one substance. In Model 2, dependence on the five substances was coded as individual categories in a single fixed factor i.e. individuals were coded as belonging to one and only one of 6 categories: "non-dependent", dependent on "alcohol", "nicotine", "cocaine", "methamphetamine", or "cannabis". Individuals in Model 2 were not allowed to be dependent on more than one substance. In both Models, MRI site was entered as a random factor while sex, age, and total intracranial volume were included as covariates. Further analyses were performed to disconfirm the existence of a site by diagnosis interaction (see Supplemental Materials). The replicability of neuroimaging results has recently been brought into question (22, 23). The large sample size of the current study facilitated the adoption of an optimized split half strategy to verify the reliability of effects. The data were split into two halves (a discovery and a replication dataset) with statistically matched stratification for age, sex and intracranial volume within each site and dependence status. Since each region-of-interest was analyzed separately, a false discovery rate method (i.e. the Benjamini-Hochberg procedure) was used to control for multiple comparisons on the first half of the data (the discovery dataset). Associations discovered in the first half of the data are reported here as significant only if they were replicated in **the**

second half of the data (the replication dataset), i.e. if the sign of the difference in means was the same and the null hypothesis had a probability of $p < 0.05$.

General versus Substance-specific Dependence Effects

Model 2 permitted a comparison of the estimated marginal mean region-of-interest volume/thickness between non-dependent controls and participants dependent on each substance. Significance was defined as in Model 1. The large impact of alcohol dependence on the data (see Results) influenced the decision to examine whether dependence on any substance except alcohol was related to differences in region-of-interest volume/thickness compared to non-dependent controls. This was assessed with a secondary linear contrast within Model 2 that grouped dependence on nicotine, cocaine, methamphetamine and cannabis (but not alcohol) in a comparison with non-dependent controls.

Past 30 Day Use

Linear mixed effects models were used to determine whether past 30 day nicotine or past 30 day alcohol use was related to the volume/thickness of regions-of-interest identified by Model 1 or 2 (i.e. those brain regions listed in Table 2). See Supplemental Information for more details.

Support Vector Machine Classification

Support vector machine classification was implemented in MATLAB (Nattick, MA: Mathworks Inc.) with a radial basis function kernel, tuned by parameter sweep in a 10-fold inner loop nested within an optimized split-half cross validation (32) (see Supplemental Methods for details). The radial basis function kernel facilitates the inclusion of non-linear relationships in the classifier. In other words, the support vector machine can detect informative patterns in the data which may not be identified by traditional linear analyses such as Model 1 and Model 2. To mitigate site, sex, age, and intracranial volume effects, region-of-interest data were residualized prior to classification. Five studies without control participants were excluded. Area-under-the-curve of the receiver operating characteristic curve, along with their corresponding *p*-values based on equivalence with the Mann-Whitney *U* test, were calculated to estimate generalizable classifier performance on the independent half of the data for each of two train-test scenarios (i.e. train on the first half, test on the second, and *vice versa*). More area-under-the-curve in a receiver operating characteristic curve, which plots true positive rate against false positive rate, indicates a better separation of the dependent and non-dependent groups. Significance for the area-under-the-curve was defined as $p < 0.05$ in both classification scenarios. The top twenty features of each classification were determined by the greatest change in cost function resulting from their individual removal from the classification (33).

Results

Demographic information is provided in Table 1 and by site in Supplemental Table 1.

Model 1: Dependent vs. Non-Dependent

Subcortical volume in dependent individuals was significantly lower in the bilateral hippocampus, bilateral amygdala and right nucleus accumbens (Table 2). Lower cortical thickness was observed in several areas including the bilateral insula, bilateral precentral gyrus, bilateral supramarginal gyrus and the right medial orbitofrontal cortex. See Table 2 for a complete list and Supplemental Table 2 for an at-a-glance summary.

Model 2: Each Substance Dependence Group Compared Separately to Non-Dependent Controls
All subcortical regions-of-interest identified in Model 1 plus the right thalamus, bilateral putamen, right globus pallidus, and left nucleus accumbens had significantly lower volume in Model 2 when alcohol dependent participants were compared to non-dependent controls. In addition, alcohol dependent participants exhibited lower average thickness in twenty-seven cortical regions-of-interest (Table 2; Figure 1). Cocaine dependence was associated with lower cortical thickness in only one brain region **(**Table 2; Figure 1). No cross-validated differences in regional volume/thickness were significant for dependence on nicotine, methamphetamine or cannabis on their own. Since most effects were related to alcohol dependence, a secondary linear contrast was performed to explore the effect of removing alcohol from the analysis. The contrast compared participants dependent on any substance except alcohol against non-dependent controls. It revealed that the insula bilaterally was significantly thinner in dependent individuals (Table 2).

Substance-specific v Shared Substance-General Effects
Three distinct patterns of results emerged which are illustrated by bar graphs in Figure 2.
Pattern 1) [Substance Specific] In most regions-of-interest where a significant difference was observed, the effect was demonstrated in Model 2 to be related specifically to dependence on alcohol alone (27 regions-of-interest), e.g. the right nucleus accumbens (Figure 2), or both alcohol and cocaine, i.e. the right supramarginal gyrus (1 region-of-interest) (Figure 1; Table 2).
Pattern 2) [Substance-General] Six cortical regions-of-interest (e.g. the left supramarginal gyrus and the right medial orbitofrontal cortex) were associated with dependence in Model 1 but were not significantly thinner in any one particular substance group relative to non-dependent controls in Model 2 (Table 2; Figures 1 & 2).
Pattern 3) [Substance-General] Two cortical regions-of-interest (i.e. the right and left insula) were significantly thinner when all dependent groups were compared to controls (Model 1), and when all dependent groups except alcohol were contrasted against controls (Model 2). In addition, the left insula was significantly thinner in the alcohol dependent group alone relative to controls (Table2; Figures1 &2).

Past 30 Day Use
The volume of several subcortical regions-of-interest were negatively associated with past 30 day use of alcohol, namely bilateral amygdala and nucleus accumbens, right hippocampus and left globus pallidus, after a false discovery rate correction for multiple comparisons. No brain regions were related to past 30 day nicotine use.

Support Vector Machine
The support vector machine produced a significant classification of alcohol and nicotine dependent individuals relative to non-dependent controls (Figure 3) in both halves of the data, $p<0.05$. The classification of cocaine dependent individuals approached significance. The top twenty structural predictors distinguishing dependence on each substance from non-dependent controls in each classification is listed in Supplemental Table 3.

Discussion

Subcortical volume or cortical thickness was significantly lower on average in dependent individuals compared to non-dependent controls across widespread parts of the brain (i.e**.** 20 distinct brain regions-of-interest out of a total of 82) (Table 2; Supplemental Table 2). Some of these differences were substance-specific while others appear to constitute a shared neural substrate associated with dependence regardless of the substance used (Figure 1). A majority of the identified regions-of-interest were smaller/thinner specifically in the brains of alcohol dependent individuals (e.g. bilateral posterior cingulate and superior frontal cortex). A more limited set of seven regions with lower cortical thickness across substance dependence groups included the bilateral insula, the left inferior parietal cortex, the right medial orbitofrontal cortex, the bilateral middle temporal cortex and the left supramarginal gyrus. No region-of-interest was significantly larger/thicker in substance dependent individuals relative to controls. An unexpected finding of the present study was the absence of substance-specific linear effects on brain volume related to nicotine, methamphetamine or cannabis dependence despite the collection of large pooled samples. Also, the successful classification of individuals dependent on nicotine, alcohol, or cocaine using the support vector machine approach suggests that the development of clinically useful neuroimaging biomarkers of substance dependence may be more productive if based on broader patterns of brain function/structure rather than differences in unique brain regions considered alone.

The set of brain regions identified with substance dependence in general are supported by prior evidence. The insula performs a central role in the perception of the internal state of the body (34). Disruption of the insula could alter regulation of the intense positive and negative bodily states associated with drug-taking and withdrawal, biasing the individual towards relapse as a maladaptive response to anticipated challenges to physiological homeostasis (35). It has been reported that smokers who have suffered brain damage involving the insula have subsequently lost the urge to smoke (36). The parietal cortex has been associated with attention and working memory (37, 38). Disruption of these processes could interfere with self-awareness about a substance use problem and the management of stressful situations. The medial orbitofrontal region-of-interest defined by Freesurfer (also known as the ventromedial prefrontal cortex) encodes the subjective value of future rewards during decision-making (39). Lesions of this brain region produce disadvantageous choices on gambling tasks that model real-life decisions (40). Altered neural activity in the insula, medial orbital and parietal cortex has frequently been linked to substance dependence and may predict greater craving and risk of relapse (41-44). The present results support the idea that substance dependence is mediated by a shared set of mechanisms across substance groups. Indeed, human twin studies suggest that vulnerability to substance dependence is accounted for principally by a shared set of genetic variations regardless of the substance used with proportionately smaller substance specific effects (16).

Although subtle in magnitude, the wide spatial distribution of alcohol specific effects is a striking finding of the study. Alcohol consumption enjoys greater cultural acceptance in the countries from which the data for this study was sampled relative to the other substances examined (45). Not only is alcohol legal to buy and consume, widely publicized government sanctioned guidelines exist for “safe” low-dose use of alcohol. This tolerance of alcohol-related health risks is unlike any of the other substances investigated here (i.e. nicotine, cocaine, methamphetamine and cannabis) whose use even in small amounts is discouraged (45). It should be noted that lifetime exposure to each substance could not be uniformly assessed in the current datasets. As a consequence, the scope of the alcohol dependence effects may, in part, be related to greater absolute consumption of alcohol relative to the other drugs. It was possible to assess past 30 day use of nicotine and alcohol, a limited proxy of level of

exposure, in a sizable minority of the datasets. Several subcortical regions-of-interest, such as the amygdala and nucleus accumbens, were significantly smaller in individuals who reported drinking the most number of alcoholic drinks in the past 30 days consistent with the notion that greater exposure could be responsible for the magnitude of the observed alcohol effects. Further studies will be required to clarify whether the greater number of observed alcohol specific effects relative to the other substances is related to differences in toxicity or total exposure.

As the first study to compare brain volume effects of dependence on five different substances and the largest neuroimaging study of addiction to date, it is also notable that, besides the seven brain regions associated with dependence in general, there were no drug specific effects for dependence on nicotine, methamphetamine and cannabis. Although cross validation demonstrated that the volumetric differences observed were reliable, the effect sizes were uniformly small (Table 2). This suggests that the lack of consistency in the literature (13-15) may be related to the insufficient power of most studies to detect true effects. Other imaging modalities such as task-based fMRI (41-44) or higher resolution structural imaging may be required to detect reliable substance-specific nicotine, methamphetamine, or cannabis effects if they exist. It is also possible that substance dependence has multiple heterogeneous interactions with brain volume that are not well assessed by simple linear analyses. Evidence for this is provided by the support vector machine classification.

The support vector machine classification found that the pattern of regional volume differences could be used successfully to distinguish between non-dependent controls and individuals dependent on alcohol and nicotine. The transformation of the data with a radial basis function kernel prior to classification facilitated the detection of non-linear patterns that cannot be detected by Model 1 and 2. Additionally, the support vector machine can identify a multivariate pattern of effects across numerous ROIs, each of which, in isolation, may not pass statistical threshold. Thus, the support vector machine detected useful information in the pattern of results that was not apparent from the linear analysis. The significant classifications suggest that the overall pattern of volumetric effects may contain useful clinical information that would not be apparent if only traditional univariate linear analyses were performed. While influential features in the classification partly overlapped with the regions-of-interest identified by the univariate analyses, e.g. brain regions associated with alcohol dependence such as the hippocampus and amygdala, additional regions not identified by the linear mixed effects analyses (i.e. Model 1 and Model 2) were also involved (Supplemental Table 3). Future efforts of the working group will include the incorporation of other imaging modalities with which it may be possible to distinguish dependence on additional substances such as methamphetamine and cannabis from non-dependent controls. It would also be clinically useful to examine whether the support vector machine classifications developed in this study offer an index of the strength of substance dependence in individuals who go on to recover or relapse. It is worth noting that current blood and urine tests do not identify dependence like the machine learning classifier in the present study but rather detect, and to an extent quantify, recent substance use. While the present findings are preliminary and the support vector machine classifications should be tested on other independent samples, if brain volume is confirmed as a viable biomarker of dependence, or biological risk of dependence, it could be used to plan how prevention and treatment resources are allocated to individual patients as well as, potentially, track intervention success. **A structural MRI scan in combination with other factors known to be related to substance use problems (e.g. change in employment or marital status, health issues) could be used to assess risk of transition to problematic patterns of use or to quantify the current degree of dependence, which would influence the intervention strategy.**

Several factors limit the interpretation of the current findings. Different diagnostic instruments were used to assess substance dependence (Supplemental Table 1). Although the validity of each of these instruments has been well established, between instrument variation could add noise to the measured behavioral phenotype. This, however, could be an advantage because the extrapolation of significant findings to the general population is also likely to be more robust by virtue of generalizing over different methods of assessment**.** The absence of nutrition and education information, which are potential confounds, also limits the interpretability of the results. A perennial concern with multi-site studies is variation attributable to different scanners and acquisition protocols. This issue was mitigated by using a standard data extraction protocol developed by the ENIGMA Project that has been validated in previous multi-site reports (20, 28-30) and the formal consideration of potential site differences in all statistical analyses. As discussed above, the degree of exposure to the various substances was not characterized uniformly across studies, which limits, for instance, the interpretation of the widespread alcohol effects and whether alcohol represents a greater source of toxicity than the other substances examined. It should be emphasized, however, that this study examined brain volumetric associations with dependence and not with total lifetime substance use. A beneficial outcome of this first study of the Addiction Working Group will be to raise awareness of the data needed to estimate the relation between brain volume and total exposure, and more generally of the utility of uniform phenotypic data for data pooling. Greater consideration of how data may be used in international collaborations may influence the collection of data in future studies, which will increase their impact beyond their primary research, focus. The PhenX toolkit (https://www.phenxtoolkit.org/), for example, provides an extensive catalog of standardized measures expressly intended to facilitate secondary cross-study comparisons. Finally, co-occurring substance use limits the interpretation of the findings. Pervasive recreational substance use is a general issue for all studies of human substance dependence. For example, it is likely epidemiologically that a methamphetamine user will be exposed to alcohol. Studying methamphetamine users who do not use any other addictive substance is an unusual group who, in practice, would be difficult to identify but, more importantly, would not be characteristic of the real-world population of methamphetamine users, i.e. there would be a selection bias. Unlike studies on animal models, it is not possible to randomly assign humans to groups with restricted exposure to one substance alone. The typical strategy, which was used in the datasets included in this study, is to screen subjects for dependence on other substances but not to exclude for non-dependent use of other substances.

The field of neuroimaging faces a crisis of relevance if published studies cannot be replicated as noted in a series of recent reviews (22, 23)**.** The authors of the present study joined together to form a working group within the pre-existing framework of the ENIGMA Project in order to assemble a sufficiently large sample to overcome issues related to low statistical power that affect most individual neuroimaging studies. Using a rigorous cross-validation method, several brain regions were found to have a reliable association with substance dependence including a shared set of regions across substances, such as the insula and the medial orbitofrontal cortex. Although the univariate analyses failed to identify linear effects in relation to nicotine, methamphetamine, or cannabis dependence specifically, a machine learning algorithm, which was also able to detect non-linear patterns in the data, successfully classified individuals dependent on alcohol or nicotine relative to non-dependent controls. This suggests that the overall pattern of volumetric effects may contain more useful information with regard to the development of a neuroimaging biomarker of substance dependence than is revealed by the magnitude of single brain regions examined in isolation.

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Tables and Figures

Table 1. Participant demographics. Differences in gender and age assessed with Pearson chi square and two sample t-test, respectively; * = $p < 0.05$.

	All Groups		Alcohol		Nicotine		Cocaine		Meth		Cannabis	
	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case
Total	1100	2140	292	898	290	602	99	227	173	228	246	185
Female	449*	731	99	291	155*	250	39*	54	71	78	85	58
(%)	(40.8)	(34.2)	(33.9)	(32.4)	(53.4)	(41.5)	(39.4)	(23.8)	(41.0)	(34.2)	(34.6)	(31.4)
Mean Age	28.5*	33.3	31.3*	34.7	26.1*	30.8	36.0*	40.2	31.7	32.9	22.7*	26.5
(+/- s.d.)	(9.9)	(10.6)	(10.2)	(10.7)	(8.0)	(9.8)	(10.3)	(7.7)	(9.3)	(10.0)	(7.5)	(10.0)

Table 2. Left and right hemisphere regions-of-interest that exhibited lower subcortical volume/cortical thickness. Names of the cortical regions-of-interest are as they appear in the Freesurfer atlas. In Model 1, all individuals were classified as either dependent or non-dependent. In Model 2, individuals were sorted by dependence on one and only one substance, i.e. individuals dependent on more than one substance were excluded from Model 2. Comparison of estimated marginal means for dependence on alcohol and cocaine relative to non-dependent controls are presented for Model 2. The additional contrast in Model 2 included individuals dependent on nicotine, cocaine, methamphetamine and cannabis (i.e. but not alcohol). Only significant associations are shown. There were no significant associations with nicotine, methamphetamine or cannabis dependence on their own.

Subcortical Volume

Model 1 Dependent v Non-Dependent

	Left *p*-value 1st half	2nd half	Cohen's *d* Both halves	Right *p*-values 1st half	2nd half	Cohen's *d* Both halves
Amygdala	0.0002	0.0042	-0.055	0.0011	0.0048	-0.041
Hippocampus	0.0000	0.0000	-0.086	0.0000	0.0000	-0.081
Nucleus Accumbens				0.0069	0.0231	-0.025

Model 2 ALCOHOL v Non-Dependent

	Left *p*-values 1st half	2nd half	Cohen's *d* Both halves	Right *p*-values 1st half	2nd half	Cohen's *d* Both halves
Amygdala	0.0000	0.0019	-0.107	0.0000	0.0003	-0.112
Globus Pallidus				0.0274	0.0006	-0.075
Hippocampus	0.0000	0.0000	-0.196	0.0000	0.0000	-0.180
Nucleus Accumbens	0.0161	0.0011	-0.048	0.0000	0.0000	-0.088
Putamen	0.0005	0.0000	-0.101	0.0001	0.0013	-0.080
Thalamus				0.0149	0.0009	-0.093

Cortical Thickness

Model 1 Dependent v Non-Dependent

	Left *p*-values 1st half	2nd half	Cohen's *d* Both halves	Right *p*-values 1st half	2nd half	Cohen's *d* Both halves
Caudal Middle Frontal	0.0000	0.0461	-0.038			
Fusiform	0.0000	0.0354	-0.035			
Inferior Parietal	0.0000	0.0355	-0.028			
Insula	0.0000	0.0003	-0.059	0.0011	0.0003	-0.044
Medial Orbitofrontal				0.0093	0.0453	-0.029
Middle Temporal	0.0101	0.0078	-0.031	0.0049	0.0470	-0.026
Paracentral Lobule	0.0022	0.0015	-0.032	0.0401	0.0061	-0.024
Precentral	0.0000	0.0024	-0.039	0.0000	0.0044	-0.042
Precuneus	0.0014	0.0415	-0.024			
Supramarginal	0.0049	0.0139	-0.028	0.0041	0.0349	-0.028

Model 2 ALCOHOL v Non-Dependent

	Left *p*-values 1st half	2nd half	Cohen's *d* Both halves	Right *p*-values 1st half	2nd half	Cohen's *d* Both halves
Caudal Middle Frontal	0.0006	0.0210	-0.064	0.0239	0.0276	-0.057
Fusiform	0.0017	0.0002	-0.074	0.0000	0.0000	-0.097
Inferior Temporal	0.0021	0.0146	-0.058	0.0148	0.0213	-0.049
Insula	0.0021	0.0000	-0.091			
Isthmus Cingulate				0.0009	0.0005	-0.079
Lateral Occipital				0.0013	0.0220	-0.042
Lateral Orbitofrontal				0.0309	0.0019	-0.064
Medial Orbitofrontal	0.0434	0.0191	-0.061			
Parahippocampal	0.0284	0.0265	-0.077			
Paracentral Lobule	0.0002	0.0001	-0.075	0.0052	0.0003	-0.062
Posterior Cingulate	0.0000	0.0000	-0.096	0.0004	0.0000	-0.089
Precentral	0.0087	0.0007	-0.063	0.0008	0.0003	-0.079
Precuneus	0.0007	0.0002	-0.065	0.0036	0.0002	-0.064
Rostral Anterior Cingulate	0.0378	0.0095	-0.085			
Superior Frontal	0.0000	0.0027	-0.075	0.0003	0.0058	-0.073
Superior Parietal	0.0187	0.0261	-0.043			
Superior Temporal	0.0228	0.0350	-0.064			
Supramarginal				0.0458	0.0165	-0.048
Temporal Pole				0.0516	0.0411	-0.063

Model 2 COCAINE v Non-Dependent

Supramarginal				0.0177	0.0490	-0.048

Model 2 Nic+Coc+Meth+Cann v Non-Dependent

Insula	0.0042	0.0348	-0.043	0.0430	0.0280	-0.034

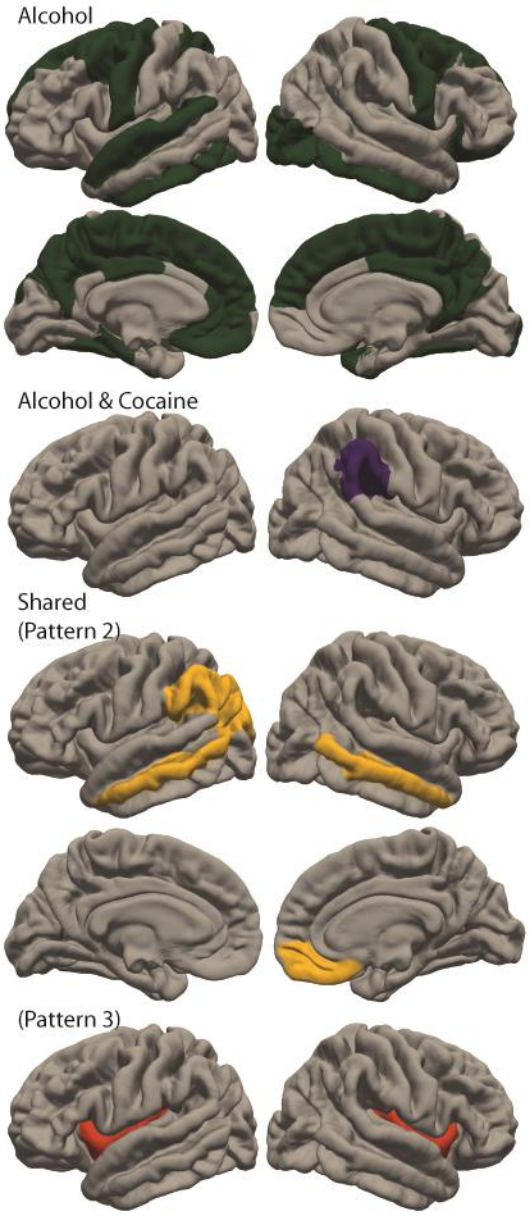

Figure 1. Cortical regions-of-interest exhibiting Substance-specific or shared Substance-General effects displayed on the surface of partially inflated average brains. Substance Specific: Alcohol alone (green), Alcohol and Cocaine (purple); Substance-General: Pattern 2 (yellow), Pattern 3 (orange).

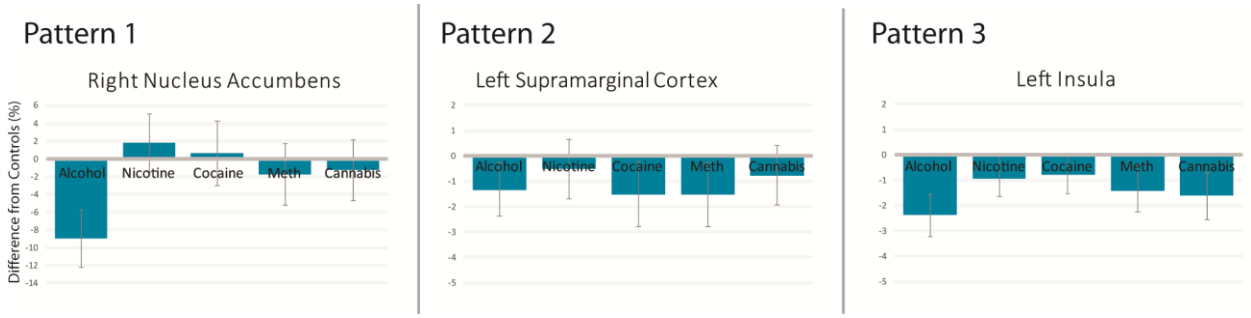

Figure 2. Dependence on the five substances studied contributed in different ways to the association of lower volume/thickness with substance dependence. Note, for illustration purposes, both halves of the data have been combined in the bar graphs. Three different patterns are illustrated: 1) Pattern 1 (substance-specific effect) lower volume in the right nucleus accumbens was largely accounted for by dependence on alcohol alone 2) Pattern 2 (substance-general effect) volume in the left supramarginal gyrus was significantly lower in dependent vs. non-dependent individuals (Model 1) but was not significantly lower in any one particular substance group (Model 2) compared to controls, and 3) Pattern 3 (substance-general effect) volume in the left insula was lower when either the alcohol dependent group or the linear contrast of all substance groups except alcohol was compared to non-dependent controls. Bars represent estimated marginal means expressed as percent difference from mean volume/thickness in non-dependent controls. Error bars represent standard error.

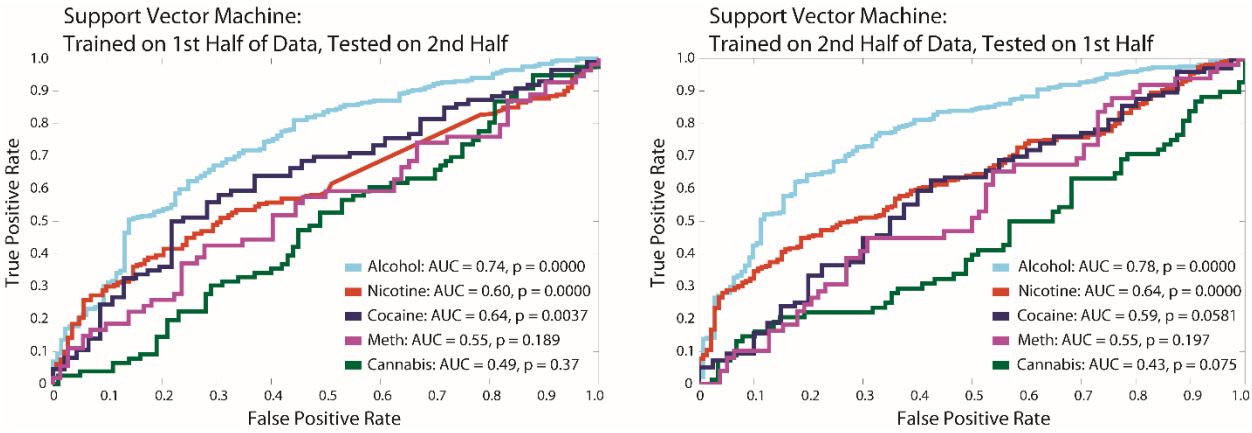

Figure 3. Plot of receiver operating characteristic curves for the support vector machine classification of individuals dependent on one of five substances relative to non-dependent controls. The area under the curve (AUC) is significant for alcohol or nicotine dependence when trained on the 1st half of the data and tested on the 2nd half (left) as well as then trained on the 2nd half and tested on the 1st half (right).