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## Severity of Alcohol Dependence is Negatively Related to Hypothalamic and Prefrontal Cortical Gray Matter Density in Heavy Drinking Smokers

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### Abstract

**Background**—While research has examined brain structure in individuals who use alcohol or nicotine, heavy drinking smokers comprise a unique subpopulation of substance users for whom less is known about the relationship between alcohol or nicotine use and structural brain abnormalities.

**Objectives**—The present study examined gray matter morphometry in a sample of 39 heavy drinking smokers (24 males, 15 females) in relation to alcohol and nicotine dependence and quantity of use.

**Methods**—Traditional voxel-based morphometry techniques were employed for preprocessing of imaging data. One multiple regression analysis for alcohol and nicotine dependence severity and another for alcohol and nicotine quantity of use were conducted, while controlling for age, gender, and total intracranial volume (ICV).

**Results**—Alcohol dependence severity was significantly negatively associated with gray matter density in the hypothalamus ( $p < 0.001$ , uncorrected) and the right superior frontal gyrus ( $p < 0.001$ , uncorrected), while controlling for nicotine dependence severity, age, gender and ICV. There were no significant relationships observed with respect to nicotine dependence severity, quantity of alcohol use, or quantity of nicotine use variables and gray matter density.

**Conclusions**—These findings suggest that within heavy drinking smokers, alcohol dependence severity is significantly related to alterations in brain structure, while this effect is not seen for quantity of alcohol or nicotine use, or severity of nicotine dependence. The current findings help clarify the contribution of alcohol and nicotine effects on brain structure, which could aid in understanding their neurocognitive consequences in heavy drinking smokers.

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## Introduction

Alcohol consumption accounts for 5.9%, or roughly 3.3 million, deaths globally each year (1). Although alcohol use alone represents a serious public health concern, high comorbidity rates have been observed at an epidemiological level between alcohol and nicotine use (2), such that 6.2 million adults in the United States endorsed both an alcohol use disorder (AUD) and dependence on nicotine (3). Moreover, an individual is three times more likely to be a smoker if he/she is dependent on alcohol and those who are dependent on nicotine are four times more likely to be dependent on alcohol (4). Given these statistics, it is evident that heavy drinking smokers comprise a distinct subpopulation of substance users that warrant unique investigation.

Magnetic resonance imaging (MRI) studies that have focused specifically on the effects that alcohol use may have on brain morphometry have investigated the relationship between drinking variables, such as lifetime duration of alcohol use (5) or lifetime alcohol intake (6, 7) and brain structure in current alcohol users. For example, Fein et al. (2002) found lifetime duration of alcohol use was negatively associated with total cortical gray matter volume in alcohol dependent males, but not in light drinkers. Moreover, findings from Taki et al. (2006) suggest a significant negative association between lifetime alcohol intake and gray matter volume reductions in the bilateral middle frontal gyri among non-alcohol dependent Japanese men. A recent study (8), however, found no significant relationship between lifetime alcohol consumption and gray matter volumes in a sample of 367 non-alcohol dependent individuals. Given these contrasting findings, it is uncertain whether quantity variables, such as lifetime alcohol intake or duration of alcohol use account for many of the gray matter volume reductions observed with continued alcohol use.

Various studies have implicated several different regions of gray matter atrophy in alcohol dependent individuals, such as the thalamus, middle frontal gyrus, insula, cerebellum, anterior cingulate cortex (ACC), and several prefrontal cortical (PFC) areas (9–11). Due to these heterogeneous results, a meta-analysis was conducted, which concluded that there were significant gray matter decreases in the ACC and left dorsal striatum/insula (9 out of 9 studies), right dorsal striatum/insula (8 out of 9 studies), and the posterior cingulate cortex (5 out of 9 studies) in alcohol dependent users relative to healthy controls (12). This suggests that brain areas implicated in processes such as reward and cognition show the most consistent gray matter atrophy in alcohol dependent individuals, but it is unclear whether overall amount of alcohol consumption or aspects of dependence severity explain these findings.

Furthermore, some of the neuroimaging studies focusing on alcohol users have not mentioned whether the alcohol users also used nicotine (5, 6), did not examine the effects of nicotine use on brain structure (7), did not control for nicotine use in their analyses (10), assessed nicotine use with a dichotomous questionnaire (8), or simply mentioned the number of smokers in the study (11). This makes it difficult to ascertain whether the observed neural effects were attributable to either alcohol and/or nicotine use and further illustrates the necessity and utility of disentangling the neural effects of each substance.

Similar to studies of alcohol use effects on brain morphometry, several MR imaging studies have been conducted to specifically examine the effects of nicotine use on brain structure (13, 14). As with studies of alcohol users, studies of cigarette smokers have attempted to quantify and incorporate a lifetime use variable, such as pack-year smoking history, which has been found to negatively correlate with PFC gray matter densities (15) as well as gray matter volume in the middle frontal gyrus, temporal gyrus, and the cerebellum (16). Interestingly, Brody et al., (2004) found no significant association between pack-year smoking history and regions of interest determined as having significant between group differences, such as the left dorsolateral PFC, ventrolateral PFC, and left dorsal ACC. Given these conflicting findings, it is uncertain whether quantity variables, such as pack-year smoking history, account for many of the gray matter volume reductions observed in nicotine dependence.

Dissimilar to studies of alcohol dependent individuals, some studies of nicotine dependent individuals have examined symptoms of dependence severity in relation to brain morphometry. For example, the Fagerström Test for Nicotine Dependence (FTND) (17), which was not associated with pack-year smoking history, was not correlated with PFC or insular gray matter density (18). The lack of a significant correlation between FTND scores and pack-year smoking history suggests that quantity of use and dependence severity symptoms may be unrelated in nicotine dependence, and thus have distinct relationships with brain structure. Overall, gray matter degradation has been observed in the thalamus, medial frontal cortex, ACC, cerebellum, and nucleus accumbens in nicotine dependent individuals (19). Due to widespread results, a meta-analysis was conducted, which found that only the left ACC showed significant gray matter reductions in nicotine dependent individuals compared to healthy controls (20).

While studying primarily alcohol or nicotine using populations carries unique benefits, specific investigation is needed into heavy drinking smokers as past studies have shown compounded neurocognitive effects (21), as well as pronounced gray matter volume reductions in heavy drinking smokers when compared to nonsmoking light drinkers (22). Chronic cigarette smoking has been found to have negative consequences on neurocognition during early abstinence from alcohol (23) and in one particular study, it was found that after 8 months of abstinence, actively smoking alcohol-dependent individuals performed worse on several neurocognitive measures, such as working memory and processing speed, when compared to never-smoking alcohol-dependent individuals (24). Additionally, formerly-smoking alcohol users were found to perform more poorly than never-smoking alcohol users at this time point. These findings not only illustrate the contribution of smoking status on neurocognitive measures but establish the clinical relevance of nicotine use in heavy drinkers. This relevance paired with the compounded neurocognitive and morphometric effects further merit investigation into this unique subpopulation of substance users.

The present work aimed to ascertain the effects of alcohol and nicotine dependence severity on gray matter density in a sample of 39 non-treatment seeking heavy drinking smokers using standard voxel-based morphometry (VBM) (25). While some imaging studies have previously investigated the relationship of FTND scores with brain structure, to our knowledge, no imaging study to date has examined how alcohol dependence severity relates

to gray matter density in heavy drinking smokers. Thus, the goal of this study was to examine if alcohol or nicotine dependence severity was correlated with gray matter density in heavy drinking smokers, while controlling for age, gender, and total intracranial volume (ICV). By examining dependence severity scores in addition to quantity of use variables, we may be able to capture how dependence is related to structural changes in the brain in a way that is not captured by variables that focus singularly on quantity of use. Based on previous findings, we hypothesized that gray matter density would be negatively related to quantity of both alcohol and nicotine use, in regions such as the middle frontal gyrus. We also hypothesized that dependence severity scores would uniquely relate to gray matter atrophy in several regions previously identified across the meta-analyses of voxel-based morphometry studies, such as the ACC, dorsal striatum, and insula.

## Methods

### Sample and Participant Selection

The subjects for the present study are a subset of participants from a medication development study of varenicline, naltrexone, and their combination in a sample of heavy drinking smokers. Subjects participated in the medication component of the study, details of which have been described in a previous publication (26), and a subsample was invited to complete a neuroimaging session. Participants were recruited from the greater Los Angeles area through online and print advertisements with the following inclusion criteria: 1) between 21 and 55 years of age; 2) reported smoking at least 7 cigarettes per day; and 3) endorsed heavy drinking per the National Institute on Alcohol Abuse and Alcoholism (NIAAA) guidelines: for men, >14 drinks per week or 5 drinks per occasion at least once per month over the last 12 months; for women, >7 drinks per week or 4 drinks per occasion at least once per month over the last 12 months. Participants were excluded from the study based on the following criteria: 1) had a period of smoking abstinence greater than 3 months within the past year; 2) reported use of illicit substances within the last 60 days, confirmed via positive urine toxicology screen at assessment visit (tetrahydrocannabinol non-exclusionary); 3) endorsed lifetime history of psychotic disorders, bipolar disorders, or major depression with suicidal ideation; 4) endorsed moderate or severe depression symptoms as measured by a score of 20 or higher on the Beck Depression Inventory-II (BDI-II) (27); 5) reported current use of psychotropic medications; 6) reported any MRI contraindications, such as any metal fragment in the body or pregnancy; and 7) reported MRI constraints, such as left-handedness or color blindness. As no Structured Clinical Interview for Diagnostic Statistical Manual 4<sup>th</sup> edition (DSM-IV), or DSM 5<sup>th</sup> edition (DSM5), Axis I Disorders (SCID-I) was administered, drinking status for participants was determined solely via NIAAA heavy drinking guidelines (28).

### Procedure

After a telephone screening to determine eligibility, participants came to the laboratory for a screening visit, during which informed, written consent was obtained. A urine cotinine test along with carbon monoxide levels verified self-reported smoking patterns and a breath alcohol concentration (BrAC) of 0.00 was required at the beginning of each visit. Eligible participants then came in for a physical examination and if eligible afterwards, began taking

medication for nine days, previously described elsewhere (26). Participants received varenicline alone (1mg, twice/day), naltrexone alone (25 mg, once/day), their combination, or matched placebo. After the medication period, participants who were eligible for the MRI session were selected at random, given an additional three days of medication, and scanned within those three days. To our knowledge, no studies to date have tested the effects of varenicline and naltrexone on structural MRI measures; however, to ensure that there were no significant gray matter differences between the medication groups, we conducted a whole-brain one-way between-subjects ANOVA (see Sample Characteristics of Results). A total of 40 subjects participated in the neuroimaging study. The Institutional Review Board of University of California, Los Angeles, (UCLA) approved all procedures for the study.

## Measures

Participants were administered the Alcohol Dependence Scale (ADS) (29), the FTND, and the 30-day Timeline Follow-back (TLFB) (30). The ADS is a 25-item self-report measure that identifies elements of alcohol dependence severity over the past 12 months, such as withdrawal symptoms and impaired control over alcohol use on a scored scale with a range of zero to 47. The FTND is a six-item self-report measure that captures features of nicotine dependence severity on a scored scale of zero to 10, and questions on this measure are not confined to a specific time frame of substance use. The TLFB assessed the daily amount of alcoholic drinks and cigarettes participants consumed in the past 30 days before the scan, from which mean drinks/drinking day (DPDD) and cigarettes/day (CPD) were calculated.

## MRI Data Acquisition and Preprocessing

All images were obtained with a 3.0 Tesla Siemens Trio MRI Scanner at the Center for Cognitive Neuroscience at UCLA. High-resolution, whole-brain sagittal structural scans were collected using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) pulse sequence with the following parameters: repetition time (TR) = 1900 ms, echo time (TE) = 2.26 ms, inversion time (TI) = 900 ms, flip angle = 9°, field of view (FOV) = 250 mm, matrix size of 256 x 256, slice thickness, 1 mm; 176 slices, and a voxel size of 1 x 1 x 1 mm<sup>3</sup>.

All structural images were processed using the VBM toolbox (VMB8, Christian Gaser, University of Jena, Jena, Germany; <http://www.neuro.uni-jena.de/vbm>) within the Statistical Parametric Mapping software package (SPM8, Wellcome Trust Centre for Neuroimaging; <http://www.fil.ion.ucl.ac.uk/spm/software/spm8>). As we expected no structural differences unrelated to gray and white matter volumes to be present in the sample, paired with past studies employing methodologies similar to ours, we chose to follow standard VBM protocols and spatially normalize the T1-weighted raw images to the same stereotactic space first (31–34). To do this, each image was registered to a standard template in Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL). After spatial normalization, the resulting DARTEL-warped T1-weighted images were segmented into three classifications (gray matter, white matter, and cerebrospinal fluid). The segmented images were then modulated, a process by which the images are multiplied by the Jacobian determinants produced for each image during spatial normalization. The advantage of modulation is that it corrects for individual

brain size and brain matter expansion or contraction that occurs during normalization. The sample homogeneity of the resulting images was checked using a mean covariance boxplot, which assesses the covariance among the sample of images across participants. Higher covariance values are preferred, which indicate the image is more similar to other volumes in the sample, while a lower covariance value signals a potential outlier (35). The mean covariance value for the current sample was .74. One participant had a covariance value (0.33) greater than 2 standard deviations from the mean. Upon inspection, the image appeared to have failed during segmentation due to motion artifact and was excluded from further analyses. This resulted in a total of 39 subjects. Finally, modulated images were smoothed using an 8-mm full width at half maximum (FWHM) Gaussian kernel. The smoothed, modulated images were used for subsequent analyses.

### Statistical Analyses

Two separate multiple regression models were built, with the first analyzing the relationship between symptoms of dependence severity and gray matter density. This model included ADS scores and FTND scores as predictor variables. The second model examined the relationship between quantity of substance use and gray matter density. The variables DPDD and CPD were chosen for this model and entered as predictor variables. Age, gender, and ICV were entered as covariates in both models. The significance level was set at  $p < 0.001$ , uncorrected with an absolute threshold mask value of 0.1, and a spatial extent threshold of 78 voxels was empirically determined per standard VBM protocol and used for analyses. Additionally, post-hoc achieved power analyses were conducted using the effect sizes calculated with Cohen's  $f^2$ .

### Follow-up analyses

Previous research has indicated that gray matter tissue can regenerate within 14 days of alcohol abstinence in alcohol dependent patients (36) and that gray matter regeneration is most profound within the first week to month of abstinence (37). Given these findings, we examined whether days to last drinking day before the imaging session correlated with gray matter density at the whole-brain level. Days to last drinking day was computed for each participant based on the TLFB information collected at the time of image acquisition. The analysis conducted included days to last drinking day as a predictor variable and age, gender, ICV, and ADS scores as covariates of interest.

Furthermore, to understand whether any of the effects were related to cannabis use within the current sample, we examined the relationship between frequency of cannabis use and drinking and nicotine variables using nonparametric Spearman's correlations. Cannabis use was assessed using a single-item categorical question asking, "On average, how often do you smoke marijuana?"

## Results

### Sample Characteristics

Means, standard deviations, and ranges are presented for participant demographics, nicotine use, and alcohol use variables in Table 1. For reference, scores of 1 to 2 on the FTND

correspond with low dependence, 3 to 4 with low to moderate dependence, 5 to 7 with moderate dependence, and 8 or more with high dependence on nicotine. Table 2 presents the means and standard deviations for alcohol and nicotine dependence and quantity variables across the four medication conditions. Analysis of variance (ANOVA) tests revealed no significant differences between the medication groups on alcohol dependence scores,  $F(3, 35) = 1.42$ ,  $p = 0.25$ , nicotine dependence scores,  $F(3, 35) = 1.28$ ,  $p = 0.30$ , quantity of alcohol use,  $F(3, 35) = 1.19$ ,  $p = 0.33$ , or quantity of nicotine use,  $F(3, 35) = 0.75$ ,  $p = 0.53$ . To ensure that there were no significant gray matter differences between the medication groups, we conducted a whole-brain one-way between-subjects ANOVA in SPM8 and found no significant regions.

### Dependence Severity Analyses

Multiple regression analyses revealed that ADS was significantly related to gray matter density in two clusters, such that higher ADS scores correlated with lower gray matter density values in these regions, while controlling for FTND scores, age, gender, and ICV (Figure 1). The first cluster corresponded to the right superior frontal gyrus (MNI coordinates of  $x = 27$ ,  $y = 45$ ,  $z = 24$ ; 415 voxels,  $p < 0.001$ , uncorrected; Cohen's  $f^2 = 0.96$ ,  $1-\beta = 0.87$ ), while the second cluster included the hypothalamus (MNI coordinates of  $x = -2$ ,  $y = -1$ ,  $z = -5$ ; 481 voxels,  $p < 0.001$ , uncorrected; Cohen's  $f^2 = 1.53$ ,  $1-\beta = 0.99$ ). Anatomical labels were determined using the Neurosynth database (Tal Yarkoni, University of Texas at Austin; <http://neurosynth.org>). Figure 2 presents the partial regression plots for both clusters with the regression line representing the relationship between ADS scores and gray matter density, after adjusting for age, gender, ICV, and FTND scores. There was no significant positive correlation with ADS scores and gray matter density and no significant positive or negative correlations with FTND scores and gray matter density.

### Quantity of Use Analyses

Multiple regression analyses revealed no significant positive or negative correlations of CPD or DPDD with gray matter density.

### Follow-up Analyses

Days to last drinking day was significantly positively correlated with gray matter density in the left postcentral gyrus, after controlling for age, gender, and ICV (MNI coordinates of  $x = -58$ ,  $y = -10$ ,  $z = 22$ ; 554 voxels,  $p < 0.001$ , uncorrected). This suggests that the longer it had been since a participant consumed alcohol, the higher the gray matter density was in this cluster. Twenty-three of the 39 participants reported any cannabis use and nonparametric correlations revealed that cannabis use was unrelated to alcohol dependence ( $\rho = 0.10$ ,  $p = 0.52$ ), nicotine dependence ( $\rho = 0.03$ ,  $p = 0.85$ ), quantity of alcohol use ( $\rho = 0.20$ ,  $p = 0.23$ ), and quantity of nicotine use ( $\rho = 0.14$ ,  $p = 0.41$ ).

### Discussion

The purpose of the present study was to examine the relationship between quantity of alcohol/nicotine use and alcohol/nicotine dependence severity with gray matter density in heavy drinking smokers. Previous studies have focused primarily on alcohol users but have

not excluded participants for nicotine use (38, 39). Similarly, some prior studies that examined nicotine users did not establish exclusionary criteria based on alcohol use (40). These studies make it difficult to ascertain whether alcohol or nicotine use/dependence account for previous findings, as their individual contributions to gray matter structure or brain activity were not examined. Thus, it is critical to investigate the unique contributions of alcohol and nicotine use to brain morphometry in heavy drinking smokers. We hypothesized that there would be gray matter reductions in areas such as the ACC, dorsal striatum, and insula. Multiple regression analyses revealed that ADS scores significantly predicted gray matter density in the hypothalamus and right superior frontal gyrus and thus, the results differed from our initial hypotheses. Contrary to our expectations, there were no significant relationships with respect to quantity of alcohol use or nicotine dependence and quantity of cigarette use variables.

The hypothalamus is part of the hypothalamic-pituitary-adrenal (HPA) axis, which has been consistently shown to be dysregulated in individuals with AUD (41, 42). HPA-axis dysregulation in alcohol-dependent individuals is marked by elevated blood glucocorticoid levels (43), which is associated with impairments in various brain regions, such as the prefrontal cortex, hippocampus, and the mesolimbic reward pathway (44). Impairments in these regions can lead to utilization of habit-based forms of learning or memory over goal-directed forms and profound cognitive memory impairments (45). The current findings indicating ADS was negatively related to hypothalamic volume in heavy drinking smokers may suggest alterations in hypothalamic gray matter density that could be associated with changes in HPA-axis functioning and related cognitive impairments. Studies that integrate measures of gray matter density, cognitive functioning and markers of HPA-axis functioning in heavy drinking smokers are needed to clarify these associations. Moreover, several studies have linked hypothalamic gray matter degradation to the presence of Korsakoff Syndrome (46, 47). These findings suggest that the development of Korsakoff Syndrome may exist on a spectrum, with hypothalamic gray matter atrophy acting as a relevant biomarker. Thus, our findings support the notion that alcohol dependence severity is related to gray matter degradation observed in the progression of uncomplicated alcoholism to Korsakoff syndrome. However, in a recent study of almost 3,000 Dutch nationals, it was demonstrated that alcohol use was associated with dysregulation in the HPA-axis system while alcohol dependence status (none, remitted, current) was not (48). Given these contrasting findings from our study, it is necessary to further explore the respective contributions of alcohol use and dependence to the dysregulation of the HPA-axis system.

The finding that higher ADS scores were negatively related to gray matter density in the superior frontal gyrus is supported by numerous previous studies indicating lower frontal gray matter density in alcohol users (5, 7, 49, 50). In a review paper discussing the construct of impulsivity, areas of the PFC, such as the ventromedial and dorsolateral PFC, were posited to be involved in the neural circuitry of delay-related decision making and inhibitory control (51). Broadly speaking, it is possible that gray matter degradation in the frontal cortex is related to behavioral inhibition and decision making deficits in alcohol dependence (52), but further research is needed to shed light on how specific features of impulsivity relate to the gray matter atrophy observed in AUD.



Various explanations can be offered as to why the results for nicotine dependence severity were nonsignificant. The FTND has fewer items than the ADS, so it is possible that lower variance of FTND scores made it difficult to detect relationships with gray matter density. It is also possible that nicotine dependence severity is not related to gray matter structure in the brain to the same extent as alcohol dependence severity. While several regions, such as the ACC, left dorsal striatum/insula, right dorsal striatum/insula, and the posterior cingulate cortex were identified as exhibiting gray matter atrophy in a meta-analysis of alcohol dependent individuals (12), a meta-analysis of chronic cigarette smokers only found the left ACC to show gray matter atrophy across several studies (20). The discrepancy may suggest differences between the two substances with respect to biological manifestations in the brain.

However, previous studies found that smoking alcohol dependent individuals had significantly decreased cortical thickness in the insula and ACC when compared to non-smoking alcohol dependent individuals (53). Additionally, heavy drinking smokers were found to have significantly smaller temporal lobe and total gray matter volumes when compared to non-smoking heavy drinkers (22). Dissimilar to those studies, quantity of nicotine use or dependence severity were not found to significantly contribute to gray matter density in the current study. Given that Durazzo, Mon, Gazdzinski, and Meyerhoff (2013) included a sample with an average FTND score of 5.4 (moderate nicotine dependence severity) and participants who smoked an average of 20 cigarettes per day, while the present sample had an average FTND score of 3.69 (low to moderate nicotine dependence severity) and participants smoked an average of 14.56 cigarettes per day, it is possible that differences in nicotine dependence severity and quantity of use between the current and previous studies explain the discrepant findings.

Previous research has found significant gray matter reduction in recovering alcohol users immediately before undergoing detoxification (54). This effect is ameliorated in abstaining light drinkers and abstaining recovering alcoholics versus relapsing recovering alcoholics (9). These findings support the notion that gray matter degradation effects could be attributable to the length of time between the last day an individual consumed alcohol and when he/she was scanned. The significant positive correlation between days to last drinking day and gray matter density in the left postcentral gyrus is consistent with the hypothesis that alcohol may cause dehydration and thus, volumetric reductions in the brain that are, in turn, ameliorated with short-term cessation of alcohol use. However, given that days to last drinking day was not related to gray matter density in the regions related to alcohol dependence severity, it is unlikely that recent alcohol use affected the current results.

While our findings demonstrate the unique contribution of alcohol dependence severity to gray matter density in heavy drinking smokers, there are various limitations that should be noted. First, there was no matched control group to the comorbid users in this study. Although the multiple regression approach permits the investigation of specific contributions of alcohol and nicotine dependence and quantity of use to gray matter density, a control group would help ascertain whether the regions identified as significantly relating to alcohol dependence severity also differ in gray matter density from healthy controls. Second, the dependence severity and quantity of use measures did not encompass the exact same time

frame, which may have resulted in relationships detected for dependence severity and gray matter density, but not quantity of use and gray matter density. However, assessing dependence severity over the past 12 months could have reflected severity over the past 30 days prior to study participation, while average DPDD or CPD in the past 30 days could have also been comparable to average DPDD or CPD over the past year. Given that the sample was comprised of non-treatment seeking participants, it is plausible to hypothesize that past month alcohol and nicotine consumption closely reflect past year consumption of these substances in the current sample. Nevertheless, as patterns of alcohol or nicotine use may vary over a longer time frame, variables that capture quantity of use or frequency of use over a longer period of time would be preferred in future studies. Lastly, given that the average FTND scores of the current participants reflected low-to-moderate nicotine dependence severity, alcohol users with more severe nicotine dependence may be required to detect effects of nicotine use on brain structure.

In conclusion, we examined the relationship between gray matter density and quantity of use/dependence severity for both alcohol and nicotine in 39 heavy drinking smokers using VBM. The multiple regression analysis revealed a significant negative relationship between ADS scores and gray matter density in two brain regions, such that higher ADS scores correlated with lower gray matter density in the hypothalamus and right superior frontal gyrus, after controlling for nicotine dependence severity, age, gender, and ICV. The current results may help clarify the contribution of alcohol and nicotine use to gray matter density in heavy drinking smokers, which could aid in understanding the neurocognitive consequences of co-morbid substance use in heavy drinking smokers.

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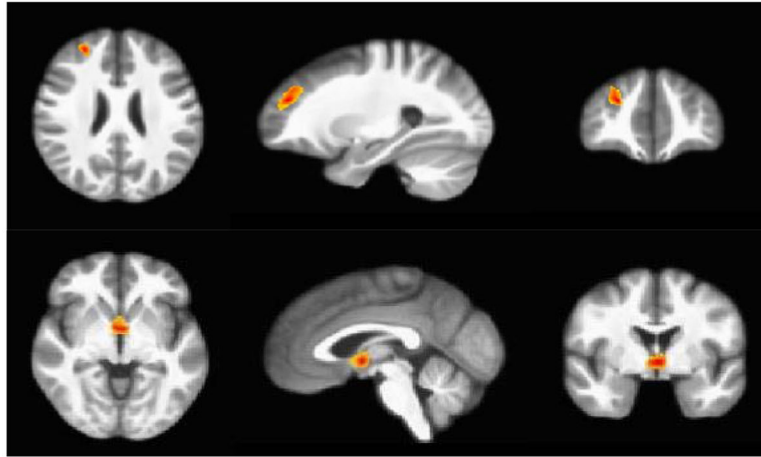
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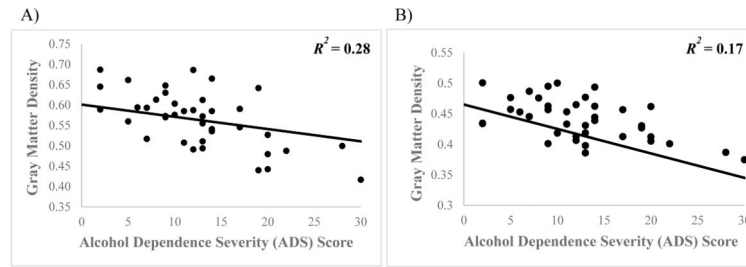
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**Figure 1.** Alcohol dependence severity is significantly negatively related to gray matter density, controlling for age, gender, ICV and Fagerström Test for Nicotine Dependence (FTND) scores. A) Superior frontal gyrus; MNI coordinates:  $x = 27, y = 45, z = 24$ , 415 voxels,  $p < 0.001$ , uncorrected; Cohen's  $f^2 = 0.96$ ,  $1-\beta = 0.87$ ; B) Hypothalamus; MNI coordinates:  $x = -2, y = -1, z = -5$ , 481 voxels,  $p < 0.001$ , uncorrected; Cohen's  $f^2 = 1.53$ ,  $1-\beta = 0.99$ .



**Figure 2.** Relationship between Alcohol Dependence Scale (ADS) scores and gray matter density in the (A) superior frontal gyrus, and (B) hypothalamus. The regression line illustrates the relationship between ADS scores and gray matter density, adjusted for age, gender, ICV, and Fagerström Test for Nicotine Dependence (FTND) scores.

**Table 1**

Demographic information, cigarette use, and alcohol use for the 39 participants included in the analyses.

Variable	All subjects
Age	31.28 (8.75) [21–50]
Gender – male/female	24/15
Ethnicity	
African-American	5/3/4
Asian	5/3/4
Caucasian	21
Latino	5/3/4
Multiracial	6
Education (years)	14.62 (3.76) [0–20]
Alcohol Dependence Scale	12.59 (6.43) [0–30]
Drinking days per month	20.54 (7.79) [3–30]
Drinks per drinking day	6.16 (3.22) [1.88–14.03]
Days to last drinking day	2.31 (1.28) [1–7]
FTND	3.69 (2.17) [0–8]
Cigarettes per day	14.56 (7.45) [7.7–40]
Smoking days per month	29.49 (1.14) [26–30]

FTND = Fagerström Test for Nicotine Dependence

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**Table 2**

Means and standard deviations across medication groups for alcohol and nicotine dependence and quantity of use indices.

	<b>VAR (N=10)</b>	<b>NTX (N=10)</b>	<b>VAR + NTX (N=10)</b>	<b>Placebo (N=9)</b>
Alcohol Dependence Scale	13.2 (5.45)	10.1 (6.12)	15.6 (8.53)	11.33 (4.24)
Fagerström Test for Nicotine Dependence	3.6 (2.46)	2.7 (1.64)	4 (2.11)	4.56 (2.3)
Drinks per Drinking Day	5.22 (3.05)	6.08 (3.27)	7.72 (3.70)	5.55 (2.61)
Cigarettes per Day	12.77 (4.41)	12.85 (5.99)	16.05 (9.65)	16.78 (8.98)

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