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Effect of Alcohol, Tobacco, and Cannabis Co-Use on Gray Matter Volume in Heavy Drinkers

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Abstract

Objective: Alcohol, tobacco, and cannabis are the three most frequently used drugs in the United States and co-use is common. Alcohol, tobacco, and cannabis use have been separately associated with altered brain structure, and alcohol and tobacco co-use results in decreases in gray matter volume. Less is known about the effect of alcohol and cannabis co-use, and alcohol, tobacco, and cannabis tri-use. Therefore, this study examined the effect of co- and tri-use on gray matter volume, a measure of brain cell density, in heavy drinkers.

Method: Heavy drinkers (n=237; 152m/85f; age=32.52; white=111; black=28; Latino=9; American Indian=2; Pacific Islander=4; Asian=59; mixed=15; other=9) were classified into four groups based on their alcohol, tobacco, and cannabis use: alcohol only users (n=70), alcohol and tobacco co-users (n=90), alcohol and cannabis co-users (n=35), and alcohol, tobacco, and cannabis tri-users (n=42). All participants completed a structural MRI scan. Voxel-based morphometry was conducted to evaluate the effect of co-use on gray matter volume, with alcohol only users as the reference group. Age, sex, and scanner were included as covariates.

Results: Alcohol and tobacco co-users had significantly decreased left orbitofrontal gray matter volume relative to alcohol only users (Cohen's d=0.79). There were no differences in gray matter volume between the alcohol only and alcohol and cannabis co-users, or between the alcohol only and tri-user groups.

Conclusions: The additive effect of tobacco co-use on gray matter volumes in heavy drinkers was limited and localized. The effect of tri-use of alcohol, tobacco, and cannabis may have

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Data Dissemination: The data in this manuscript represent a combination of structural neuroimaging data collected by our lab. Some of the functional neuroimaging data, which use the structural images as a reference, have been previously reported (Courtney, Ghahremani, & Ray, 2013; Courtney & Ray, 2014; Cservenka, Courtney, Ghahremani, Hutchison, & Ray, 2017; Grodin, Ray, MacKillop, Lim, & Karno, 2019; Lim et al., 2019; Ray et al., 2015). However, none of the aforementioned studies examined structural brain changes, and this study represents a unique combination of these data sets.

interacted, such that overlapping cannabis and tobacco use masked volume differences present in separate co-using groups.

Keywords

voxel-based morphometry; alcohol; cannabis; tobacco; co-use

Introduction

Alcohol, tobacco, and cannabis are the three most commonly used substances in the United States (Substance Abuse and Mental Health Services Administration [SAMHSA], 2016). Moreover, co- and tri-use of alcohol, tobacco, and cannabis is common (Falk, Yi, & Hiller-Sturmhöfel, 2006; Moss, Chen, & Yi, 2014), and the use of alcohol, tobacco, or cannabis independently increases the probability of co-use of the two remaining substances (Roche et al., 2019). Individuals with a 12-month or lifetime diagnosis of alcohol use disorder (AUD) have an increased likelihood of tobacco dependence and an increased likelihood of having another substance use disorder (Grant et al., 2015). Similarly, co-users of cannabis and alcohol were found to be at heightened risk of having AUD and cannabis use disorder (CUD) (Blanco et al., 2016; Weinberger, Platt, & Goodwin, 2016; Winters & Lee, 2008), and cannabis and tobacco co-use is associated with greater likelihood of meeting diagnostic criteria for both cannabis and tobacco dependence (Rubinstein, Rait, & Prochaska, 2014).

A growing body of literature has highlighted the adverse consequences associated with alcohol and tobacco co-use. Heavy-drinking tobacco smokers have worse health outcomes than alcohol or tobacco smokers alone (Hart, Davey Smith, Gruer, & Watt, 2010; Xu et al., 2007), and have a faster cognitive decline relative to non-smoking moderate drinkers (Hagger-Johnson et al., 2013). Moreover, alcohol and tobacco co-users have a lower likelihood of successful smoking cessation (Kahler et al., 2010), and smoking lapses occur more frequently when individuals are drinking alcohol, especially during heavy drinking days (Kahler, Spillane, & Metrik, 2010). Similarly, co-use of alcohol and cannabis is quite common (Agrawal, Lynskey, Madden, Bucholz, & Heath, 2007; Midanik, Tam, & Weisner, 2007). Co-users of alcohol and cannabis report greater use of both substances, have a greater likelihood of an AUD, and have more negative consequences compared to single substance users (Martin, Kaczynski, Maisto, & Tarter, 1996; Midanik et al., 2007; Sokolovsky, Gunn, Micalizzi, White, & Jackson, 2020). Additionally, alcohol and cannabis co-use negatively impacts clinical outcomes, including increased risk of meeting criteria for a comorbid psychiatric disorder, heavy drinking, and poorer prognoses for AUD treatment (Metrik, Gunn, Jackson, Sokolovsky, & Borsari, 2018; Mojarrad, Samet, Cheng, Winter, & Saitz, 2014; Subbaraman, Metrik, Patterson, & Swift, 2017; Yurasek, Aston, & Metrik, 2017).

In addition to a host of health and clinical correlates, abnormalities in brain structure have been shown in alcohol, tobacco, and cannabis users compared to healthy controls. Brain volume loss is well-documented in individuals with AUD. Consistent and significant gray matter reductions are found in corticostriatal-limbic circuits, with areas including the bilateral insula, superior temporal and frontal gyri, striatum, dorsolateral prefrontal cortex (DLPFC), precentral gyrus, anterior and posterior cingulate cortices (ACC/PCC), nucleus

accumbens, thalamus, and hippocampus (Grodin & Momenan, 2017; Li et al., 2019; Xiao et al., 2015; X. Yang et al., 2016). Additionally, volume reductions in these brain areas have been related to alcohol use factors, such that right striatum volume is negatively correlated with duration of alcohol dependence, and left frontal cortex and thalamus gray matter atrophy is related to lifetime alcohol consumption (Cardenas, Studholme, Gazdzinski, Durazzo, & Meyerhoff, 2007; X. Yang et al., 2016).

Current smokers show gray matter atrophy in similar regions. Meta-analyses of smoking studies showed consistent regional gray matter volume decrease in the prefrontal cortex, insula, and ACC (Pan et al., 2013; Z. Yang, Zhang, Cheng, & Zheng, 2020; Zhong et al., 2016) as well as the olfactory gyrus, with correlations between pack-years and gray matter loss in the prefrontal cortex and ACC (Fritz et al., 2014). Gray matter density was shown to be lower in the left prefrontal cortex in high pack-years smokers, and density was inversely correlated with pack-years (Zhang et al., 2011). However, some studies found the converse, with gray matter volume increase in the right lingual cortex (Zhong et al., 2016) and left occipital cortex (Z. Yang et al., 2020), and increased gray matter density in the left insular cortex (Zhang et al., 2011) of smokers compared to controls.

Cannabis use has been associated with gray matter volume reductions in the amygdala and hippocampus (Cousijn et al., 2012; Koenders et al., 2016; Weinstein, Livny, & Weizman, 2016) as well as medial temporal cortex, parahippocampal gyrus, insula, and orbitofrontal cortex (Battistella et al., 2014). These regions are functionally implicated in motivational, emotional, and affective processing, and are known to be rich in cannabinoid CB1 receptors (Burns et al., 2007). However, cannabis users also showed increased gray matter volume in basal ganglia regions including the caudate, putamen, pallidum, and nucleus accumbens, compared to controls (Moreno-Alcázar et al., 2018). Furthermore, THC/CBD ratio was shown to inversely correlate with gray matter volume in the right hippocampus, possibly indicating some protective effects of CBD and neurotoxic effects of THC (Demirakca et al., 2011). In brief, the literature on the effects of cannabis on gray matter volume is mixed.

While gray matter abnormalities have been well-studied in these three substances separately, relatively few studies have explored these structural changes in the context of substance co-use. Tobacco and alcohol co-use is the most well-studied form of substance co-use, with studies finding consistent brain volume decreases in smoking heavy drinkers compared to non-smoking heavy drinkers, in both total neocortical gray matter (Durazzo, Gazdzinski, & Meyerhoff, 2007) and specifically frontal, temporal, and parietal gray matter volume (Durazzo, Cardenas, Studholme, Weiner, & Meyerhoff, 2007). Furthermore, alcohol-dependent smokers and non-smokers both demonstrated smaller volumes than non-smoking light-drinking controls in most cortical regions of interest, with volume differences between alcohol-dependent smokers and non-smokers only seen in the putamen (Durazzo et al., 2014). Taken together, these results indicate important interaction effects of tobacco and alcohol co-use. A recent study of substance co-use found a negative correlation between the number of substances used (alcohol, tobacco, cannabis, and cocaine) and gray matter volume of the medial prefrontal cortex, indicating that alterations may not be substance-specific but may be related to the number of substances used, while other regions (including gray matter reductions in the thalamus associated with tobacco use and ventrolateral

prefrontal cortex associated with cocaine) showed substance-specific effects. (Kaag et al., 2018). Another study exploring polysubstance use disorder (alcohol and at least two other substances) showed volume reductions in the thalamus compared to healthy controls, as well as polysubstance users showing volume increases in the right caudate compared to participants with alcohol dependence only (Grodin & Momenan, 2017). Tobacco smokers, cannabis users, and tobacco-cannabis co-users all exhibited increased gray matter volume in the putamen compared to healthy controls, while tobacco smokers and tobacco-cannabis co-users showed decreased gray matter volume in the thalamus (Wetherill et al., 2015). Finally, though not a study of co-use, a mega-analysis of individuals with dependence on any one of five substances (alcohol, nicotine, cannabis, cocaine, or methamphetamine) found that participants with any substance use disorder had lower subcortical volume in bilateral hippocampus, amygdala, and right nucleus accumbens. Some regions were substance-specific (i.e., alcohol-specific deficits in the right nucleus accumbens), while others, including volume reductions in the left supramarginal gyrus and insula, were substance-general, indicating that differential alterations in gray matter volume may underlie different cognitive effects associated with various substance use disorders (Mackey et al., 2019).

No studies to date have explored differences in gray matter volume between the co- and tri-use of alcohol, tobacco, and cannabis and single-substance use. Thus, in the current study, we compare gray matter volume differences between heavy drinking subjects who solely drank alcohol against those who were alcohol and tobacco co-users, alcohol and cannabis co-users, and alcohol, tobacco, and cannabis tri-users. As the literature regarding co-use and polysubstance use suggests a complex interplay between substance use and regional volume increases and decreases, we did not have a directional hypothesis for this study. Instead, we used a data-driven, voxel-based morphometry approach to localize the effect of co- and tri-substance use on gray matter volume.

Methods:

Data source and sample:

The current sample is culled from seven separate clinical and experimental psychopharmacology neuroimaging studies with similar inclusion and recruitment methods, all conducted in the Addictions Laboratory at the University of California, Los Angeles. The study sample was drawn from studies examining the neural correlates of risk-taking and alcohol administration (Courtney, Ghahremani, & Ray, 2013; Courtney & Ray, 2014), the neural correlates of alcohol prediction error (Cservenka, Courtney, Ghahremani, Hutchison, & Ray, 2017), the effect of a brief intervention on neural response to alcohol taste cues (Grodin, Ray, MacKillop, Lim, & Karno, 2019), and four pharmacotherapy trials investigating the effect of naltrexone (Lim et al., 2019), the combination of and naltrexone and varenicline (Ray et al., 2015) and (Ray et al., under review), and ibudilast (Grodin et al., under review). Although some studies involved pharmacological manipulations, all demographic and clinical characteristics analyzed herein were collected at a baseline assessment visit (prior to medication randomization or any experimental procedures). All studies recruited community samples of non-treatment-seeking drinkers from the greater Los

Angeles Area. All study procedures were approved by the University of California, Los Angeles Institutional Review Board, and all participants provided written informed consent after receiving a full explanation of the study procedures.

Heavy drinking was verified through one of the following methods: (1) diagnosis of alcohol dependence (DSM-IV-Tr) or alcohol use disorder (DSM-5); (2) greater than 7 drinks per week for women and greater 14 drinks per week for men; (3) an Alcohol Use Disorder Identification Test (AUDIT; Saunders, Aasland, Babor, de la Fuente, & Grant, 1993) score of 8 of higher; or (4) drinking at binge levels (4 or more drinks per episode for women, 5 or more drinks per episode for men) 4 or more times in a month. All studies had the following exclusion criteria: (1) current involvement in treatment programs for alcohol use or have received treatment in the prior 30 days to study participation; (2) use of psychoactive drugs (excluding cannabis) or use of prescription medications for recreational purposes; (3) lifetime diagnosis of schizophrenia, bipolar disorder, or psychotic disorders; (4) current use of mood stabilizers, sedatives, anti-anxiety medications, seizure medications, or prescription painkillers; (5) self-reported history of contraindicated medical conditions (e.g., chronic liver disease, cardiac disease); (6) if female, pregnant (as verified by a urine sample), nursing, or planning to get pregnant in the next 6 months or refusal to use a reliable method of birth control; (7) breath alcohol concentration (BrAC) of greater than 0.000 g/dl; and (8) positive urine toxicology screen for any drug (other than cannabis). Additionally, all studies had the following exclusion criteria for neuroimaging: (1) history of epilepsy, seizures, or severe head trauma; (2) claustrophobia; and (3) non-removable ferromagnetic objects in body.

Clinical Battery:

Across studies, all participants completed a phenotypic battery consisting of sociodemographic (i.e., age, sex, years of education, race and ethnicity, and estimated income) and clinical assessments capturing harmful and hazardous alcohol drinking (AUDIT; Saunders et al., 1993), alcohol use disorder severity (Alcohol Dependence Scale; ADS; Skinner, Horn, & Addiction Research Foundation of, 1984), alcohol-related problems (The Drinker Inventory of Consequences; DrInC; Miller, Longabaugh, National Institute on Alcohol, & Alcoholism, 2000), alcohol craving (Obsessive-Compulsive Dependence Scale; OCDS; Anton, Moak, & Latham, 1995 & Penn Alcohol Craving Scale; PACS; Flannery, Volpicelli, & Pettinati, 1999), tobacco dependence (Fagerstrom Test of Nicotine Dependence; FTND Heatherton, Kozlowski, Frecker, & Fagerstom, 1991), and cannabis use severity (Cannabis Use Disorder Identification Test; CUDIT; Adamson et al., 2010). Interview based assessments captured alcohol withdrawal symptoms (Clinical Institute Withdrawal Assessment for Alcohol Scale, Revised; CIWA-Ar; Sullivan, Sykora, Schneiderman, Naranjo, & Sellers, 1989) and a 30-day Timeline Followback; TLFB; Sobell & Sobell, 1992), which assessed alcohol drinking, tobacco smoking, and cannabis use over the past month. From the TLFB the following indices were calculated: total drinks, drinks per day (DPD), drinks per drinking day (DPDD), heavy drinking days (HDD; indicated by ≥5 drinks/day for men, and ≥4 drinks/day for women), total tobacco smoked, and number of days of cannabis use. The Structured Clinical Interview of DSM-IV-Tr (SCID) or DSM-5 was administered by a master's level clinician to determine age at first drink and assess for current AUD and substance use disorder (SUD) symptoms. In order to

facilitate (and streamline) the merging of data across multiple studies using both DSM-IV and DSM-5 criteria, diagnoses from the DSM-IV were transformed to the DSM-5 use disorder classification, such that participants who were diagnosed with alcohol dependence using DSM-IV terminology were considered to have an AUD

Co-Use Classification:

Participants were classified into one of four groups based on their substance co-use behavior (ascertained via questionnaires and face-to-face interviews): (1) alcohol use only (AO); (2) alcohol and tobacco co-use (AT); (3) alcohol and cannabis co-use (AC), and (4) alcohol, tobacco, and cannabis use (ATC). Individuals in the AO group had a negative urine toxicology screen and reported no tobacco or cannabis use on the 30-day TLFB. These reports were corroborated by data analyses of the FTND, CUDIT, and SCID substance use disorder module. Individuals in the AT group had a negative urine toxicology screen and reported alcohol and tobacco use on the 30-day TLFB, which was corroborated by the FTND. This group reported no cannabis use on the 30-day TLFB and reported no use on the CUDIT and SCID SUD module. Finally, individuals in the ATC group reported alcohol, tobacco, and cannabis use on the 30-day TLFB and had a positive urine toxicology screen for THC. Individuals in the ATC group also reported tobacco use on the FTND and cannabis use on the CUDIT and/or in the SCID SUD module. Of note, not all of the studies from which data was culled for the present study collected the CUDIT and/or FTND; as such, corroborating data was used when available. See Table 1 for descriptive statistics and sample size for each measure.

MRI Acquisition:

Scanning took place at the UCLA Center for Cognitive Neuroscience on one of two scanners: (1) a 3.0T Siemens Magnetom Trio Scanner (4 studies); or (2) a 3.0T Siemens Prisma scanner (3 studies). Regardless of study, a structural scan was acquired for registration to the study-specific functional data. Specifically, T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2,530 ms, TE = 1.74 ms, time to inversion = 1,260 ms, flip angle = 7°, voxel size: 1 mm3, FOV = 256 mm^2, ~6.2 minutes) was collected for each participant. For all studies, at the start of the scanning visit, participants were required to have a BrAC of 0.000 g/dL and a urine toxicology screen negative for all drugs (excluding cannabis), and women were required to have a negative pregnancy test.

Data Analysis:

To compare the four groups on gray matter volume, a series of analyses of variance (ANOVAs) were conducted as omnibus tests comparing the co-use groups on continuous demographic and clinical measures. Similarly, chi-square tests were used to compare co-use groups on categorical measures. Tukey-Kramer t-tests were used to follow-up significant omnibus ANOVAs to identify the specific group differences. Group comparison analyses were conducted in SPSS 26.

Neuroimaging structural data was analyzed with FSL-VBM (Douaud et al., 2007; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized VBM protocol (Good et al., 2001)

conducted through the FSL library of analysis tools (Smith et al., 2004). First, participants' structural images were brain-extracted and gray matter segmented before being registered to the MNI 152 standard space using non-linear registration (Andersson, Jenkinson, & Smith, 2007). Next, the registered images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. The study-specific template was created for each comparison (i.e., 3 study specific templates were created). Second, all native gray matter images were non-linearly registered to the study-specific template and modulated to correct for local expansion or contraction due to the non-linear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 4 mm. Finally, a series of voxelwise general linear modes were applied using permutation-based non-parametric testing, correcting for multiple comparisons across space. Five thousand permutations were run for each comparison. For all VBM comparisons age, biological sex, and scanner (Magnetom Trio or Prisma) were included as covariates. Years of education was explored as a covariate but was non-significant in all comparisons. Effect size (Cohen's *d*) was calculated for significant results.

Results:

Co-Use Classification:

A total of 251 individuals had structural MRI scans and completed the clinical battery. Fourteen participants were not included in the final analyses due to inconsistent reporting of alcohol, tobacco, and cannabis use (n=10) or poor MRI data quality (n=4). Therefore, the final analyzed sample consisted of a total of 237 participants. Of the final sample, 70 individuals were classified as alcohol only (AO; 29.54%), 90 individuals were classified as alcohol and tobacco co-users (AT; 37.97%), 35 individuals were classified as alcohol and cannabis co-users (AC; 14.77%), and 42 individuals were classified as alcohol, tobacco, and cannabis co-users (ATC; 17.72%). The groups differed on a host of alcohol use variables, including drinking quantity and frequency (TLFB), alcohol use severity, and alcohol craving. Generally, the AC group reported the lowest amount of drinking and lowest severity levels. The 2 tobacco smoking groups (AT and ATC) did not differ on 30-day tobacco use or on tobacco dependence. The 2 cannabis using groups (AC and ATC) differed on the number of days of cannabis use, but not on cannabis use severity (see Table 1 for full breakdown).

Voxel-Based Morphometry Results

Alcohol Only vs. Alcohol + Tobacco Co-Users—There was a significant group difference in gray matter volume in the left orbitofrontal cortex (OFC; peak coordinates: x = −28, y = 30, z = −22; 162 voxels) between the AO and AT groups, such that the AO group had significantly larger volumes compared to the AT group ($p = 0.046$; see Figure 1). There were no regions where AT had larger gray matter volumes compared to AO ($p > 0.05$). There were also no sex differences between AT and AO groups ($p > 0.05$).

Alcohol Only vs. Alcohol + Cannabis Co-Users—There were no significant group differences between the AO and AC co-users in gray matter volume after controlling for age, sex, and scanner type ($p > 0.05$)[1]. There were no sex differences between AC and AO groups ($p > 0.05$).

Alcohol Only vs. Alcohol + Tobacco + Cannabis Tri-Users—There were no significant group differences between the AO and ATC tri-users in gray matter volume ($p > 0.05$). There were also no sex differences between ACT and AO groups ($p > 0.05$).

Discussion

This study examined the effect of co- and tri-use of alcohol, tobacco, and cannabis on gray matter volume in heavy drinkers. Despite widespread group differences on demographic and clinical measures, gray matter differences were highly localized and sparse. Specifically, the alcohol only group had larger left orbitofrontal gray matter volumes relative to alcohol and tobacco co-users. There were no significant group differences in gray matter between the alcohol only group and alcohol and cannabis co-users, or the alcohol only group and the alcohol, tobacco, and cannabis tri-users.

The decreased left orbitofrontal gray matter volume in the alcohol and tobacco co-users relative to alcohol only users is supported by previous work in this field. Durazzo et al. (2007) found that smoking heavy drinkers had smaller frontal gray matter volumes than non-smoking light drinkers, while non-smoking heavy drinkers had similar frontal gray volumes relative to non-smoking light drinkers, indicating that smoking combined with heavy drinking leads to additional frontal gray matter volume deficits. A study investigating the effect of polysubstance use in alcohol, tobacco, and cocaine users found a negative dose-response association between the number of substances used and medial orbitofrontal gray matter volume (Kaag et al., 2018). Smoking also impacts cortical thickness in the orbitofrontal cortex; tobacco smokers had thinner left medial orbitofrontal cortices than never smokers after controlling for alcohol intake (Kühn, Schubert, & Gallinat, 2010). Gray matter volume of the left OFC is also associated with interactions between smoking and the rs1137070 polymorphism of monoamine oxidase A (Shen et al., 2019). The orbitofrontal cortex is involved in many processes implicated in addictive disorders, including executive function. While not examined in the present study, previous work has found poorer executive function in smokers with AUD relative to non-smokers with an AUD (Durazzo, Rothlind, Gazdzinski, Banys, & Meyerhoff, 2006), indicating that chronic tobacco smoking worsens neurocognition in AUD (Glass et al., 2006). Regarding clinical characteristics, the alcohol only and alcohol and tobacco co-users differed on some alcohol use measures, including the ADS, where alcohol and tobacco co-users had higher alcohol dependence severity than the alcohol only group. However, the groups did not differ on heavy drinking days or drinks per drinking day in the past month, indicating that the groups were engaging in similar patterns of recent heavy drinking, and did not differ in measures of alcohol craving or drinking-related consequences. However, this study was unable to examine the effect of cumulative use, which may have differed between the alcohol only and alcohol and tobacco co-users.

There were no significant differences between the alcohol only group and the alcohol and cannabis co-users, after controlling for age, biological sex, and scanner type. The literature

[1]When sex was not included as a covariate, there was a significant group difference in gray matter volume in the left lateral occipital cortex (peak coordinates: x = −28, y = 30, z = −22; 235 voxels) between the AO and AC groups, such that the AO group had significantly larger volumes compared to the AC group ($p = 0.03$).

on the effects of cannabis use on gray matter volume is mixed, with some studies finding increased gray matter volume in cannabis users (Moreno-Alcázar et al., 2018) and others finding reductions Weinstein, Livny, & Weizman, 2016). Interestingly, a group difference was originally seen, such that the alcohol only group had larger gray matter volume in the left lateral occipital cortex than the alcohol and cannabis co-use group; however, this difference did not remain significant when controlling for sex. Therefore, we recommend that future studies examine potential sex interactions with alcohol and cannabis co-use. The alcohol only and alcohol and cannabis co-using groups did not differ significantly on alcohol frequency or quantity measures (e.g., TLFB indices), or on alcohol use severity measures (e.g., AUD diagnosis, AUDIT and ADS scores). The groups differed on alcohol craving and drinking consequences, such that the alcohol only group reported higher alcohol craving on the OCDS and higher total DrInC scores. This result adds to the mixed literature on cannabis and alcohol co-use, which largely suggests that co-use of alcohol and cannabis results in an additive effect of poorer mental health and substance use outcomes (Agrawal et al., 2007; Blanco et al., 2016; Midanik et al., 2007; Weinberger et al., 2016; Yurasek et al., 2017). However, these effects have not been consistently represented within this body of literature (Mallett, Turrisi, Trager, Sell, & Linden-Carmichael, 2019). It is also important to note that participants from the studies from which these data were culled generally represent primary alcohol users, as these studies specifically recruited heavy drinkers. As such, it is possible that the alcohol-only group displayed higher levels of craving and increased alcohol-related problems as a result of a ceiling effect, such that the effects of drinking-related consequences were more pronounced in the alcohol only group.

There were no significant group differences in gray matter volume between the alcohol only users and the alcohol, tobacco, and cannabis tri-users. The lack of volume deficits in the tri-use group may be due to an interaction between cannabis and tobacco. While gray matter volume decreases are well-established in alcohol and tobacco co-users (Durazzo et al., 2007; Durazzo et al., 2007; Durazzo et al., 2014), tobacco and cannabis co-use has been associated with gray matter volume increases (Wetherill et al., 2015), and cannabis users alone have shown increased subcortical gray matter volumes (Moreno-Alcázar et al., 2018). Therefore, the interacting effects of tobacco and cannabis on gray matter volume may have resulted in the present finding of no group differences between the alcohol only and the tri-using groups. Importantly, the tri-using group reported greater cannabis use than the alcohol and cannabis co-using group, while reporting similar tobacco smoking and nicotine dependence severity as the alcohol and tobacco co-using groups. Further complicating this interaction is the evidence for a potential neuroprotective effect of cannabidiol (CBD) and a neurotoxic effect of tetrahydrocannabinol (THC) (Demirakca et al., 2011). In the present study, cannabis use was recorded as a binary variable at each day (i.e., yes/no use on a given day) and therefore, we were unable to evaluate the THC/CBD ratio in the cannabis co-using groups. Additionally, this study did not include individuals with a cannabis use disorder (CUD). The effects cannabis and the interactive effects of alcohol, tobacco, and cannabis on gray matter volume may only become present in individuals with higher severity of use.

The present study must be evaluated based on its strengths and weaknesses. Study strengths include the sample sizes of the single, co-, and tri-use groups and the use of a data-driven, voxel-based morphometry approach to investigate gray matter deficits. Among the study

limitations are the lack of a non-heavy drinking control group, the lack of detailed collection on cannabis use, including cannabis strain, potency of THC, and modality of delivery, and the relatively young age of the sample. Of note, this study was unable to examine the effect of years of alcohol use on gray matter volume due to missing data. Moreover, this was a cross-sectional study and cannot identify if gray matter volume differences are a cause or a consequence of use or co-use. Detailed longitudinal studies will be needed to interrogate the temporal precedence of structural brain changes. Relatedly, this study and many other structural neuroimaging studies did not control for socioeconomic factors, as this information was not collected from all participants, which may affect brain development. Finally, this study did not explicitly exclude individuals with depressive or anxiety disorders, which may also impact gray matter volume.

In conclusion, this study found only small, localized differences in gray matter between alcohol only users and alcohol and tobacco co-users. There were no significant group differences in gray matter volume between alcohol only users and alcohol and cannabis co-users, or between alcohol only users and alcohol, tobacco, and cannabis tri-users. Given that the effects of substance co-use, particularly cannabis, is poorly understood, this study adds to the body of literature by providing a well-phenotyped sample with high-quality voxel-based morphometry assessments. The pattern of findings does not suggest a detriment in gray matter volume for alcohol and cannabis co-users in this sample; however, additional research with more severe samples is required to fully elucidate these effects and investigate the potential neuroprotective properties of cannabis.

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Public Health Significance:

- This study highlights the importance of investigating co- and tri-use of tobacco and cannabis in heavy alcohol users.
- This study indicates that the effect of co-use of alcohol and tobacco on the brain in heavy drinkers is localized when compared to heavy alcohol users alone.
- This study indicates that the effect of cannabis and alcohol co-use did not affect brain volume when compared to heavy alcohol users alone.

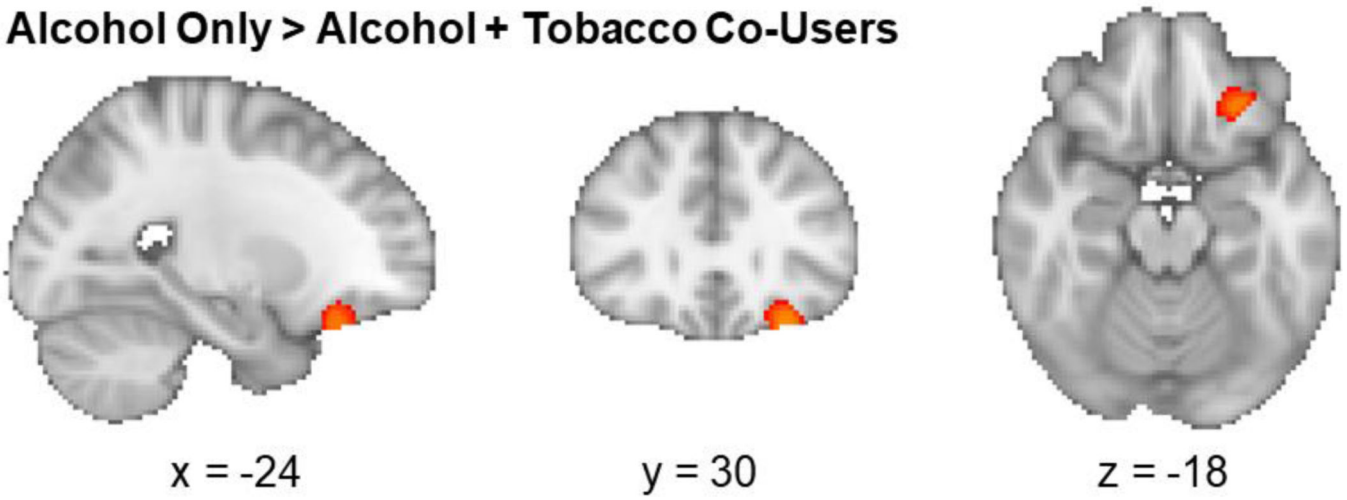

Figure 1.

Alcohol and tobacco co-users had decreased gray matter volume in the left orbitofrontal cortex (significant voxels shown in red [dark gray]) relative to alcohol only users (p=0.046, corrected).

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Table 1.

Demographic and Clinical Characteristics

Variable Mean ± SD or n (%)	Alcohol only (n = 70)	Alcohol + Tobacco (n = 90)	Alcohol + Cannabis (n=35)	Alcohol + Tobacco + Cannabis (n=42)	Statistic	p
Age [d, g]	**30.74 ± 9.18**	**34.42 ± 10.46**	**28.11 ± 7.26**	**35.07 ± 12.59**	**F = 4.89**	**0.003**
Sex (Male)	36 (51.42%)	64 (71.11%)	24 (68.57%)	28 (66.67%)	$X^2 = 7.23$	0.07
Education (years) [e]	**14.54 ± 2.30**	**14.92 ± 2.24**	**14.63 ± 2.51**	**13.55 ± 3.34**	**F = 2.86**	**0.04**
Estimated Income	**n=48**	**n=76**	**n=27**	**n=33**	**$X^2 = 39.95$**	**0.02**
<$15,000	5 (7.14%)	17 (18.89%)	2 (5.71%)	14 (33.33%)		
$15–000-$29,999	12 (17.14%)	21 (23.33%)	8 (22.86%)	6 (14.29%)		
$30–000-$44,999	3 (4.29%)	12 (13.33%)	5 (14.29%)	5 (11.90%)		
$45–000-$59,999	3 (4.29%)	10 (11.11%)	1 (2.86%)	3 (7.14%)		
$60–000-$74,999	5 (7.14%)	4 (4.44%)	3 (8.57%)	2 (4.76%)		
$75–000-$89,999	6 (8.57%)	6 (6.67%)	3 (8.57%)	2 (4.76%)		
$90–000-$104,999	2 (2.86%)	3 (3.33%)	1 (2.86%)	0 (0%)		
$105–000-$119,999	3 (4.29%)	1 (1.11%)	0 (0%)	1 (2.38%)		
>$120,000	9 (12.76%)	2 (2.22%)	4 (11.42%)	0 (0%)		
Race/Ethnicity					$X^2 = 27.88$	0.14
White	37 (52.76%)	45 (50%)	10 (28.57%)	19 (45.23%)		
Black	5 (7.14%)	11 (12.22%)	5 (14.29%)	7 (16.67%)		
Latino	1 (1.42%)	6 (6.67%)	2 (5.71%)	0 (0%)		
American Indian	1 (1.42%)	1 (11.11%)	0 (0%)	0 (0%)		
Pacific Islander	0 (0%)	2 (2.22%)	0 (0%)	2 (4.76%)		
Asian	21 (30%)	16 (17.78%)	15 (42.86%)	7 (16.67%)		
Mixed	3 (4.29%)	6 (6.67%)	1 (2.86%)	5 (11.90%)		
Other/Unknown	2 (2.86%)	3 (3.33%)	2 (5.71%)	2 (4.76%)		
Hispanic/Latino	25 (35.71%)	18 (20%)	9 (25.71%)	15 (35.71%)	$X^2 = 6.21$	0.10
Total Drinks (30 Day TLFB) [a, c, d, g]	**87.27 ± 66.55**	**120.23 ± 89.99**	**72.33 ± 42.26**	**130.15 ± 94.88**	**F = 5.70**	**0.001**
Drinking Days (30 Day TLFB) [a, d]	**16.61 ± 7.20**	**19.66 ± 7.13**	**15.71 ± 6.73**	**19.76 ± 7.60**	**F = 4.43**	**0.005**
DPDD (30 Day TLFB) [g]	**5.26 ± 2.62**	**5.99 ± 3.02**	**4.72 ± 1.81**	**6.46 ± 3.48**	**F = 3.23**	**0.02**

Variable Mean ± SD or n (%)	Alcohol only (n = 70)	Alcohol + Tobacco (n = 90)	Alcohol + Cannabis (n=35)	Alcohol + Tobacco + Cannabis (n=42)	Statistic	p
HDD (30 Day TLFB) [d, g]	**8.81 ± 7.38**	**11.38 ± 8.21**	**6.77 ± 5.05**	**11.93 ± 9.60**	**F = 4.26**	**0.006**
Current AUD (%) [c, f]	**55 (78.57%); n = 70**	**43 (86%); n = 50**	**26 (76.47%); n = 34**	**42 (100%); n =42**	**x^2 = 8.99**	**0.03**
AUDIT [c, g]	**11.93 ± 9.78; n = 56**	**14.18 ± 9.09; n = 44**	**9.31 ± 7.73; n = 35**	**16.83 ± 9.44; n = 42**	**F = 4.84**	**0.003**
ADS [a, b, d, e]	**21.13 ± 15.11; n = 48**	**29.55 ± 14.02; n = 66**	**10.96 ± 5.06; n = 27**	**15.63 ± 6.50; n = 19**	**F = 16.36**	**<0.001**
AUD Symptom Count	4.69 ± 2.79; n = 52	5.13 ± 2.54; n =39	4.45 ± 1.99; n = 20	5.05 ± 2.42; n = 37	F = 0.47	0.71
CIWA-Ar	0.73 ± 1.27; n = 70	0.82 ± 1.56; n = 50	0.31 ± .63; n = 35	.043 ± .831; n= 42	F = 1.80	0.15
PACS [d]	**13.95 ± 7.58; n = 65**	**15.67 ± 7.63; n =90**	**10.97 ± 8.13; n=35**	**14.88 ± 6.16; n = 40**	**F = 3.39**	**0.02**
OCDS [b, d]	**20.28 ± 13.56; n = 43**	**21.04 ± 13.20; n = 27**	**12.54 ± 8.18; n = 26**	**19.12 ± 10.78; n = 17**	**F = 2.86**	**0.04**
DrInC [b, d]	**56.24 ± 31.04; n = 59**	**61.74 ± 34.46; n = 31**	**36.09 ± 26.33; n =23**	**57.31 ± 24.24; n = 16**	**F = 3.50**	**0.02**
Total Tobacco (30 Day TLFB)	-	332.24 ± 254.96; n = 84	-	305.40 ± 281.41; n = 42	T = 0.12	0.73
FTND	-	3.76 ± 2.27; n = 88	-	3.93 ± 2.26; n = 40	T = 0.001	0.99
Total Cannabis Days (30 Day TLFB) [f]	-	-	**6.20 ± 7.49**	**12.43 ± 11.85**	**T = 15.79**	**<0.001**
CUDIT	-	-	6.00 ± 3.53; n = 15	6.57 ± 3.26; n = 42	T = 0.53	0.48

[a] AO < AT

[b] AO > AC

[c] AO < ATC

[d] AT > AC

[e] AT > ATC

[f] AT < ATC

[g] AC < ATC

AO = alcohol only; AT = alcohol and tobacco co-user; AC = alcohol and cannabis co-user; ATC = alcohol, tobacco, and cannabis tri-user; TLFB = Timeline Followback; DPDD = drinks per drinking day; HDD = heavy drinking days; AUD = alcohol use disorder; AUDIT = Alcohol Use Disorder Identification Test; ADS = Alcohol Dependence Scale; CIWA-AR = Clinical Institute Withdrawal Assessment for Alcohol Scale, Revised; PACS = Penn Alcohol Craving Scale; OCDS = Obsessive Compulsive Drinking Scale; DrInC = Drinker Inventory of Consequences; FTND = Fagerstrom Test of Nicotine Dependence; CUDIT = Cannabis Use Disorder Identification Test