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# Shared Predisposition in the Association Between Cannabis Use and Subcortical Brain Structure

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# Abstract

**Importance** Prior neuroimaging studies have suggested that alterations in brain structure may be a consequence of cannabis use. Siblings discordant for cannabis use offer an opportunity to use cross-sectional data to disentangle such causal hypotheses from shared effects of genetics and familial environment on brain structure and cannabis use.

**Objectives** To determine whether cannabis use is associated with differences in brain structure in a large sample of twins/siblings and to examine sibling pairs discordant for cannabis use to separate potential causal and predispositional factors linking lifetime cannabis exposure to volumetric alterations.

**Design, Setting, and Participants** Cross-sectional diagnostic interview, behavioral, and neuroimaging data were collected from community sampling and established family registries from August 2012 to September 2014. This study included data from 483 participants (22-35 years old) enrolled in the ongoing Human Connectome Project, with 262 participants reporting cannabis exposure (ie, ever used cannabis in their lifetime).

**Main Outcomes and Measures** Cannabis exposure was measured with the Semi-Structured Assessment for the Genetics of Alcoholism. Whole-brain, hippocampus, amygdala, ventral striatum, and orbitofrontal cortex volumes were related to lifetime cannabis use (ever used, age at onset, and frequency of use) using linear regressions. Genetic ($\rho_g$) and environmental ($\rho_e$) correlations between cannabis use and brain volumes were estimated. Linear mixed models were used to examine volume differences in sex-matched concordant unexposed (n = 71 pairs), exposed (n = 81 pairs), or exposure discordant (n = 89 pairs) sibling pairs.

**Results** Among 483 study participants, cannabis exposure was related to smaller left amygdala (approximately 2.3%; $P = .007$) and right ventral striatum (ap-

of left amygdala (approximately 2.5%; $P = .007$) and right ventral striatum (approximately 3.5%; $P < .005$) volumes. These volumetric differences were within the range of normal variation. The association between left amygdala volume and cannabis use was largely owing to shared genetic factors ($\rho_g = -0.43$; $P = .004$), while the origin of the association with right ventral striatum volumes was unclear. Importantly, brain volumes did not differ between sex-matched siblings discordant for use (fixed effect = −7.43; $t = -0.93$, $P = .35$). Both the exposed and unexposed siblings in pairs discordant for cannabis exposure showed reduced amygdala volumes relative to members of concordant unexposed pairs (fixed effect = 12.56; $t = 2.97$; $P = .003$).

**Conclusions and Relevance** In this study, differences in amygdala volume in cannabis users were attributable to common predispositional factors, genetic or environmental in origin, with little support for causal influences. Causal influences, in isolation or in conjunction with predispositional factors, may exist for other brain regions (eg, ventral striatum) or at more severe levels of cannabis involvement and deserve further study.



## Introduction

Cannabis is the most widely used recreational drug in developed nations.[1] Its legal status has been a source of enduring controversy,[2] gaining particular momentum in the United States.[3] Yet, concerns about putative influences of cannabis on brain structure/function remain salient.[4] Neuroimaging studies have found inconsistent evidence linking cannabis to brain structure where previous meta-analyses noted possible associations between cannabis and hippocampus (and potentially amygdala) structure.[5,6]

Small sample sizes in prior studies (generally N < 100) and varied definitions of cannabis exposure may have contributed to these inconsistent findings. Addi-

tionally, studies have implied that cannabis causes volumetric alterations, despite typically not controlling for potential confounding effects of shared predispositional factors (eg, genes/rearing environment that contribute to both volumetric differences and cannabis use). For instance, Gilman et al[7] compared recreational cannabis users (n = 20) with matched nonusers (n = 20) and posited, based on observed dose-dependent relationships, that larger left ventral striatal (VS) gray matter density was likely a consequence of cannabis use. However, such cross-sectional case-control designs cannot account for the possibility that volumetric variations might predate cannabis use and/or might relate to cannabis use via predispositional factors, even in a dose-dependent manner.

Longitudinal studies, particularly with assessments preceding onset of cannabis use, are ideally suited to disentangle the effects of cannabis on the developing brain from preexisting differences. Such study designs have shown that smaller orbitofrontal cortex (OFC) volumes were associated with later cannabis initiation in adolescents[8] yet also find emerging deficits in white matter as a consequence of heavy alcohol and cannabis use.[9] However, even in cross-sectional studies, twins/siblings discordant for cannabis exposure provide a unique opportunity for differentiating predispositional/familial factors from causal effects of cannabis on the brain. As monozygotic (MZ) twins share all genetic material identical by descent, neural differences between MZ twins discordant for cannabis exposure can be potentially attributed to the causal effects of cannabis.[10,11] In contrast, if no differences are found, then large causal effects of cannabis on the brain are unlikely and, instead, the association might be attributed to genetic factors/familial environment. For instance, Gilbertson and colleagues[12] found that posttraumatic stress disorder severity in combat-exposed veterans negatively correlated with their hippocampal volume and the volumes of their noncombat-exposed MZ cotwins, implicating predispositional rather than causal mechanisms. In contrast, Lessov-Schlaggar et al[13] found functional differences in VS response to reward and punishment in regular tobacco smokers when compared with their nonregular-smoking MZ cotwin, implying potential causal mechanisms. Impor-

tantly, although data from MZ twins are essential to demonstrate causality in this manner, the absence of neural differences within discordant dizygotic (DZ) twin or nontwin sibling pairs is still compelling evidence against a causal hypothesis. If volume differences are not observed among discordant pairs who share only 50% of their genes, then finding an association with further genetic matching (ie, MZ pairs) would be unlikely.[10]

The goal of the current study was to test previously observed relationships between cannabis and brain volumes in a large normative sample of twins/siblings from the Human Connectome Project (HCP; N = 483). First, we examined whether cannabis exposure, age at onset of use, and lifetime frequency of use were associated with whole-brain volume (WBV) and amygdala, hippocampus, VS, or OFC volumes. Second, we quantified the degree to which shared genetic and individual-specific environmental factors contributed to these associations. Finally, to test whether any significant volumetric differences could be attributed to predispositional/familial or causal factors, we compared volumes across sex-matched twin/sibling pairs (henceforth referred to as *sibling pairs*) discordant for cannabis exposure (n = 89 pairs), concordant for exposure (n = 81 pairs), or concordantly unexposed (n = 71 pairs).

# Methods

## Participants

Participants were drawn from the September 2014 public data release from the HCP (N = 527), which aims to recruit 1200 individuals (3-4 siblings per family, most including a twin pair).[14] All participants were aged 22 to 35 years; for all inclusion/exclusion criteria, see the study by Van Essen et al.[14] Participants were not excluded for recreational drug use (unless they reported being hospitalized for ≥2 days for substance abuse or being treated by a medical specialist for ≥12 months for substance abuse [or any psychiatric or neurological condition]). Institutional review board approval for this study was obtained from Washington

University in St Louis, and all patients provided written informed consent that included permission for public release of the data.

Participants were excluded from the current analyses if they lacked good-quality structural magnetic resonance imaging data (n=17), were missing relevant interview/questionnaire data (n=11), or if they screened positive for tetrahydrocannabinol on a urine screen but reported not using cannabis within the last 12 months (n=16). This resulted in a final sample size of 483 individuals (262 having ever used cannabis). In analyses involving sibling pairs (eAppendix 1 in the **Supplement**), 36 individuals who did not have a full sibling or twin in the present data release were excluded. Because sex is significantly related to both cannabis use and brain volumes, the inclusion of discordant opposite-sex nontwin sibling pairs (all twin pairs were of the same sex) can result in statistical confounds. Therefore, we excluded 145 opposite-sex pairs, resulting in 241 sibling pairs (50 MZ, 45 DZ, and 146 nontwin siblings [mean sibling age difference, 3.69 years]), including 89 same-sex pairs discordant for cannabis exposure, 81 concordant for cannabis exposure, and 71 concordantly unexposed pairs (eTable 1 in the **Supplement**).

## Brain Volume Data

High-resolution (0.7-mm isotropic voxels) anatomical images were acquired using a customized Siemens Skyra 3-T scanner with a 32-channel head coil. For details on data acquisition and preprocessing, see the study by Glasser et al[15] (see eAppendix 2 in the **Supplement** for relevant preprocessing steps). Volume estimates for the regions of interest were extracted using FreeSurfer version 5.3.0 (http://surfer.nmr.mgh.harvard.edu/).[16,17] This included WBV (total gray+cortical white matter volume) and left and right amygdala, hippocampus, and VS volumes from subcortical segmentation and OFC volumes (lateral+medial) from cortical parcellation using the Desikan et al[18] atlas.

## Questionnaire, Interview, and Behavioral Data

### Cannabis Use

Cannabis-related measures were assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA).[19] Cannabis exposure was a dichotomous variable representing whether an individual reported ever using cannabis during their lifetime. Age at onset of cannabis use (reported age at first cannabis use) and lifetime frequency of use (reported number of times using cannabis across one's lifetime) were also examined (both coded ordinally by the HCP; eAppendix 3 in the **Supplement**).

### Covariates

The main analyses controlled for demographic factors of sex (female > male), race/ethnicity (white > not; African American > not), age, zygosity (MZ > not; DZ > not), total household income (eAppendix 3 in the **Supplement**), and age-adjusted picture vocabulary scores as a proxy for crystallized intelligence[20] (eAppendix 4 in the **Supplement**)—henceforth, these are referred to as *primary covariates*. Follow-up regression analyses controlled for a variety of other potential confounds including personality, impulsivity, other substance use, and comorbid psychopathology (eTable 2 in the Supplement). See eAppendix 5 in the **Supplement** for rationale and assessment details.

## Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 20 (IBM Corp) and R version 3.1 (The R Project for Statistical Computing).[21] One outlier (>3 × the interquartile range away from the 25th/75th percentile) for right amygdala and right hippocampal volume was Winsorized to the next most extreme value. Independent-sample *t* tests and $\chi^2$ tests were used to test for differences in the covariates between cannabis-exposed and unexposed individuals.

### Regression Analyses

Linear regressions were used to test whether cannabis exposure (in the full sample, N = 483) and age at onset or frequency of use (among exposed individuals

ple, N = 483) and age at onset or frequency of use (among exposed individuals, n = 262) were associated with WBV or subcortical (left and right amygdala, hippocampus, and VS) or OFC volumes, controlling for the primary covariates and WBV (when predicting regional volumes). Bootstrapped 95% CIs were calculated to account for familial clustering (R boot package[22]). False-discovery rate was used to control for the 9 regression analyses with each cannabis measure. Only brain regions with $q < 0.05$ were examined in the sibling analysis.

## Sources of Variance and Covariance

Sequential oligogenic linkage analysis routines (SOLAR)[23] were used to attribute phenotypic correlations ($\rho_p$) between cannabis exposure and brain volumes to overlapping genetic ($\rho_g$) or individual-specific environmental ($\rho_e$) factors.[24] All models controlled for the primary covariates and WBV (when examining regional volumes).

## Discordant Sibling Analyses

All possible same-sex sibling pairs were drawn from the data (n = 241 pairs from 146 families; details in eAppendix 1 in the **Supplement**). Linear mixed models (lmer function in R package lme4[25]) were used, which nested individuals within sibling pairs and nested pairs within families. The mixed models included fixed effects for the primary covariates and WBV (when predicting regional volumes). The main effect of interest was membership in a sibling pair concordant or discordant for cannabis exposure. To test this, participants were divided into 4 groups: individuals from concordant unexposed pairs, individuals from concordant exposed pairs, exposed members of discordant pairs, and unexposed members of discordant pairs. This 4-group factor was tested as a fixed effect using Helmert contrast coding (eTable 3 in the **Supplement**).

**Figure 1** displays hypothetical patterns of results from the linear mixed model analyses that could be found by the 3 contrasts examined. Contrast 1 compared exposed and unexposed siblings from discordant pairs to test a causal hypothe-

sis, ie, cannabis causes altered brain volumes (depicted as smaller volumes for exposed vs unexposed individuals in **Figure 1**A). Siblings share 50% of their genes and much of their rearing environment. Therefore, within-pair volumetric differences would be preliminary evidence for causation, pending replication in MZ pairs. Contrasts 2 and 3 both hypothesize no volumetric differences between the exposed and unexposed members of the discordant pairs and thus tested facets of the hypothesis that cannabis use and brain volumes share predispositional factors. This would suggest that differences in volume likely predate (or co-occur with) cannabis use and that other variables, such as genetic liability or rearing environment, may lead to both neural differences and liability to cannabis use. Alternatively, these other factors could contribute to neural differences, in turn increasing liability to cannabis use. Contrast 2 compared brain volumes from concordant exposed pairs with both members of discordant pairs to test exposure-related differences by concordance/discordance (**Figure 1**B). A significant effect might indicate that concordantly exposed pairs are at greater liability for cannabis use and altered brain volumes (because both siblings have used cannabis) than discordant pairs (ie, graded liability). Contrast 3 compared volumes from concordant unexposed pairs with all other groups to test whether altered brain volumes and cannabis exposure were associated with a shared predisposition (**Figure 1**C). A significant effect here implies that both concordant exposed and discordant pairs are at the same genetic liability regardless of whether one or both siblings use cannabis.

## Control Analysis

To confirm that differences in brain volumes among discordant pairs in the linear mixed models (contrast 1) were nonsignificant owing to familial matching rather than a reduction in sample size, we randomly paired each cannabis user from a same-sex discordant pair (n = 89) with a sex-matched unrelated unexposed individual. Paired *t* tests were performed to compare volumes for these unrelated pairs, ie, to remove the familial control while matching for the sample size.

# Results

## Sample Characteristics

Of the 483 participants, 262 (54.2%) reported ever using cannabis. Forty-nine percent of the exposed individuals reported first using cannabis by age 17 years and 29.4% reported using cannabis more than 100 times (eFigure in the **Supplement**). Cannabis users were significantly more likely to be male; be nonwhite; report lower income; report greater alcohol, cigarette, and other illicit drug use; report more childhood conduct problems; be less agreeable and more impulsive; and show steeper delay discounting compared with never users (**Table 1**). Similar relationships were also observed with increasing frequency of use and decreasing age at onset (eTable 4 in the **Supplement**). Descriptive statistics and intercorrelations among brain volumes are presented in eTable 5 in the **Supplement** and intercorrelations among all covariates are in eTable 6 in the **Supplement**.

## Regression Analyses

**Table 2** summarizes the linear regressions results, controlling for the primary covariates (full results in eTables 7-9 in the **Supplement**). Relative to unexposed individuals, cannabis users had smaller left amygdala (approximately 2.3%) and right VS (approximately 3.5%) volumes. Post hoc comparisons showed similar volumetric differences in those who used fewer than 100 or 100 or more times, with no statistical difference between the regression coefficients across these levels of use (eTable 10 in the **Supplement**). Among cannabis users, heavier use was associated with smaller left hippocampus volumes (although this effect did not pass false-discovery rate correction). There were no significant associations with age at onset of use (eTable 9 in the **Supplement**). These results remained significant when also controlling for a variety of personality factors, impulsivity, other substance use, and psychiatric history (eTable 2 in the **Supplement**).

## Sources of Variance and Covariance

The heritability of cannabis exposure (ever vs never used) was estimated at

67.2% (standard error, 13.6%; $P < .001$; **Table 3**). All brain volumes of interest were also significantly heritable. There was no evidence for contributions of shared rearing environment. As previously noted, only the left amygdala ($\rho_p = -0.175$; $P = .005$) and right VS ($\rho_p = -0.154$; $P = .02$) showed significant phenotypic correlations with cannabis use. Decomposing these correlations, we found a significant genetic correlation between left amygdala volume and cannabis use ($\rho_g = -0.433$; $P = .004$) but not a significant environmental correlation ($\rho_e = 0.280$; $P = .19$). Neither genetic factors nor environmental factors were significant for right VS volume.

## Discordant Sibling Analyses

Given these results, we focused on the left amygdala and right VS for the sibling analyses. Contrast 3 was significantly associated with left amygdala volumes ($\beta = 12.56$; $t_{302.80} = 2.97$; $P = .003$), supporting the predispositional hypothesis. We did not find evidence for either the causal (contrast 1) or graded liability (contrast 2) hypotheses. Concordant unexposed pairs had larger amygdala volumes than all other groups, even unexposed members of discordant pairs (**Figure 2**). The estimated marginal means (SEs) for each group were as follows: 1534.502 (14.829) for concordant unexposed, 1487.364 (15.500) for discordant unexposed, 1472.501 (15.449) for discordant exposed, and 1492.870 (13.957) for concordant exposed (**Figure 2**).

Despite not using cannabis, these unexposed individuals from discordant pairs had amygdala volumes resembling cannabis-exposed individuals, including their exposed siblings. None of the 3 contrasts significantly were associated with right VS volumes (fixed effects predicting all volumes are presented in eTable 11 in the **Supplement**).

Results including opposite-sex sibling pairs are presented in eTable 12 in the **Supplement**. We observed significant effects of contrast 3 (predisposition), con-

trast 1 (causal), and an interaction between contrast 1 and pair-sex concordance, underscoring the confounding effect of sex, within pairs, with respect to cannabis use and brain volume.

### Control Analyses

Finally, the exposed members of the same-sex discordant pairs (n = 89) were compared with unrelated but sex-matched unexposed individuals. Significantly lower volumes for the left amygdala (approximately 5.1%) were observed for the exposed vs unexposed members of these unrelated pairs (eTable 13 in the **Supplement**). This confirmed that the lack of volumetric differences between exposed and unexposed members of discordant pairs (contrast 1) was attributable to familial matching and not a reduced sample size.

## Discussion

### Cannabis Exposure Findings

To our knowledge, this is the largest study to date examining the association between cannabis exposure (ever vs never used) and brain volumes. Cannabis exposure was associated with smaller left amygdala and right VS volumes; these findings persisted even after controlling for a host of covariates. While other studies have noted reductions in amygdala volumes,[26-28] the right VS finding is somewhat unique to this study. It contradicts the increased VS volume in occasional cannabis users reported by Gilman and colleagues.[7] It remains to be explored whether this is owing to the cannabis measures explored (eg, cannabis exposed vs joints per week), sample size (483 vs 40), or other sample characteristics (eg, race/ethnicity; 74.5% white in our sample but unreported by Gilman et al[7]).

Importantly, by leveraging the familial design of the HCP, we demonstrated that amygdala volumetric reductions among cannabis users are primarily attributed to familial factors shared by twins/siblings. Overlapping genetic factors ($\rho_g$)

were the only significant source of covariance; a significant correlation between individual-specific environmental factors ($\rho_e$) would be expected if the causal hypothesis were supported.[29] The discordant sibling analyses further confirmed this; even in the absence of cannabis exposure, smaller amygdala volumes were observed among individuals with heightened familial liability, given their sibling's cannabis use. However, interpretations from our sibling analyses should be tempered by our limited sample size to examine discordant MZ pairs only. This predisposition to smaller brain volumes, even in the absence of manifest cannabis use, casts considerable doubt on hypotheses that cannabis use, at least at the levels noted in this sample, causes reductions in amygdala volumes. Instead, both the exposed and unexposed siblings in discordant pairs and concordant exposed pairs tended to have smaller amygdala volumes than concordant unexposed individuals.

## Limitations and Future Directions

First, while the normative sampling of the HCP is a strength, we were limited by sample size from examining heavy/problematic cannabis use, which has been inconsistently associated with volumetric alterations.[27,30] Furthermore, because heavy/problem cannabis use is often associated with psychiatric problems,[31] severity and chronicity of use may have been limited by the HCP's exclusion of individuals receiving extended psychiatric treatment or hospitalization. Also, data were not available on recent duration/frequency of use, which has been linked to structural changes.[32] Thus, although we noted similar evidence of association with lighter (<100 times) and heavier (≥100 times) lifetime cannabis exposure, we were underpowered to test this in our sibling analyses and could not exclude the role of causal factors at higher levels of cannabis exposure. Second, although right VS volume and cannabis use were significantly related, we were unable to disentangle the etiology of this relationship. Neither genetic nor environmental correlations were significant and within-pair differences could be equated across all groups. Thus, causal and/or predispositional influences may link cannabis exposure and VS volume. Third, while the family structure in the

HCP data are powerful, longitudinal data that were collected from prior to cannabis onset through later development is critical for substantiating causal claims (eg, National Institutes of Health efforts[33]). Fourth, the small sample size of discordant MZ twin pairs (n = 9 pairs) is a limitation. Fifth, the role of additional covariates cannot be excluded (eg, childhood trauma is linked to both amygdala volumes[34] and cannabis use[35]). Sixth, while we selected a priori regions of interest based on prior studies, other regions should be examined in future work (eg, using whole-brain voxelwise analysis). Seventh, exploring other brain-related measures, such as white matter integrity and task-related activity, might reveal different findings. Eighth, evidence for potential causal effects in opposite-sex sibling pairs was driven by a small number (n = 14) of discordant pairs where the female sibling was the exposed member. A thorough examination of phenotypic data failed to distinguish these discordant exposed females from others in the sample. However, based on evidence for sex differences in the endocannabinoid system,[36,37] such differences in related individuals may reflect qualitatively different pathways and warrants further study. Finally, future work should further explore the graded liability hypothesis.

## Conclusions

Despite speculation regarding the neurotoxic effects of tetrahydrocannabinol based on preclinical research (eg, studies by Scallet[38] and Landfield et al[39]), the observed cannabis-related volumetric differences were well within the range of normal variation. When using a simple index of exposure (ie, ever vs never use), we found no evidence for the causal influence of cannabis exposure on amygdala volume. Future work characterizing the roles of causal and predispositional factors underpinning neural changes at various degrees of cannabis involvement may provide targets for substance abuse policy and prevention programs.

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## Article Information

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*Study concept and design:* Pagliaccio, Barch, Bogdan, Heath, Agrawal.

*Acquisition, analysis, or interpretation of data:* All authors.

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