Original Investigation

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

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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 (ρ_g) and environmental (ρ_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 (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.

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Editorial

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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. Additionally, 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. Importantly, 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 χ^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, 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 (ρ_p) between cannabis exposure and brain volumes to overlapping genetic (ρ_g) or individual-specific environmental (ρ_e) factors.[24] All models controlled for the primary covariates and WBV (when examining regional volumes).

Discordant Sibling Analyses

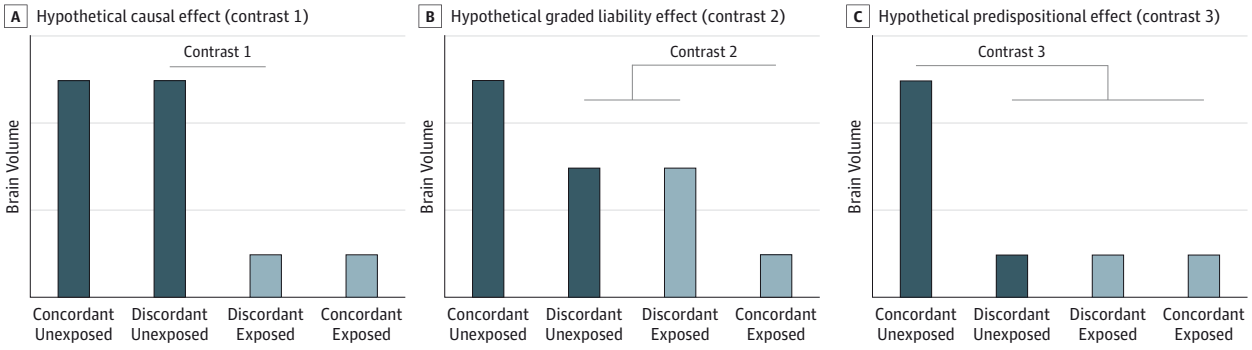
All possible same-sex sibling pairs were drawn from the data (n = 241 pairs from 146 families; details in eAppendix 1 in the Supplement). Linear mixed models (lmer function in R package lme4[25]) were used, which nested individuals within sibling pairs and nested pairs within families. The mixed models 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 hypothesis, ie, cannabis causes altered brain volumes (depicted as smaller volumes for exposed vs unexposed individuals in Figure 1A). 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 1B). 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 1C). 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

Figure 1. Hypothetical Causal and Predispositional Effects



Three hypothetical patterns of results from the linear mixed model analyses are presented here. A, The hypothesis that cannabis causes alterations in brain volumes (depicted as reductions in volume) was tested with contrast 1. As denoted by the bar labeled contrast 1, this contrast tested for differences between exposed and unexposed members of pairs discordant for cannabis use. Finding smaller volumes among exposed members of these pairs compared with their siblings would support this causal hypothesis (pending replication with monozygotic twin pairs). B, Contrast 2 compares volumes for concordant exposed pairs with discordant pairs to test the hypothesis that brain volumes and cannabis use share familial/predispositional factors where concordant exposed pairs are at increased liability (both siblings are exposed vs only one), ie, graded liability. C, Contrast 3 compares volumes for the concordant unexposed pairs with all other groups to test the hypothesis that brain volumes and cannabis use share familial/predispositional factors but that liability does not differ by concordance vs discordance for use.

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).

Table 1. Sample Characteristics of 483 Participants Included From the September 2014 Data Release of the Human Connectome Project[a]

Characteristic	Cannabis, Mean (SD)	
	Unexposed (n = 221)	Exposed (n = 262)
Age, y	29.30 (3.46)	29.32 (3.43)
Total household income[b]	5.24 (2.10)	4.82 (2.21)
Age-adjusted picture vocabulary	108.42 (14.57)	106.35 (19.99)
NEO-FFI		
Contentiousness	34.92 (5.55)	34.60 (5.66)
Extraversion	30.18 (6.21)	30.81 (6.22)
Neuroticism	16.66 (7.32)	16.26 (6.99)
Openness	27.37 (5.78)	28.42 (6.29)
Agreeableness[b]	32.59 (4.66)	31.60 (4.72)
Delay discounting (AUC)[c]	0.28 (0.22)	0.21 (0.16)
ASR impulsivity[b]	1.14 (1.18)	1.40 (1.30)
Female, %[c]	69.68	52.29
White,%[c,d]	79.64	70.23
Twins, %	53.85	46.56
Monozygotic twins, %	50.42	43.44
Lifetime depression history, %	8.60	7.25
Any childhood conduct problems,%[b]	33.94	42.75
Times ever used illicit drugs, %[c]	2.71	34.35
≥2 Alcoholic drinks per day (heaviest), %[c,e]	63.35	88.17
Never smoked cigarettes (heaviest), %[c,e]	90.95	57.63
Cannabis use		
Age at onset <17 y, %	NA	48.85
Lifetime quantity of use >11 times, %	NA	51.15
Current abuse/dependence diagnosis, %	NA	17.56
Use in the past 12 mo, %	NA	36.26

Abbreviations: AUC, area under the curve; ASR, Achenbach Adult Self-Report; NA, not applicable; NEO-FFI, NEO Five-Factor Inventory.

[a] Independent-samples *t* tests were used to compare means for the exposed and unexposed groups. χ^2 Tests were used to compare ordinal/binary variables across groups. eAppendix 5 in the Supplement provides further details.

[b] $P < .05$.

[c] $P < .001$.

[d] Race/ethnicity was coded as white, black or African American, or other race/ethnicity (including Asian, Pacific Islander, Native Hawaiian, multiracial, other, or not reported).

[e] Alcoholic drinks per day and heaviness of cigarette smoking variables refer to use during the 12-month period of heaviest use.

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), contrast 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 (ρ_g) were the only significant source of covariance; a significant correlation between individual-specific environmental factors (ρ_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

Table 2. Regression Results for the Association Between Cannabis-Related Measures and Brain Volumes[a]

	Cannabis Exposed vs Unexposed (n = 483)				Lifetime Amount of Use Among Exposed Individuals (n = 262)			
	Regression Coefficients				Regression Coefficients			
Volume	Unstandardized (b)	Standardized (β)	*P* Value	Bootstrapped 95% CI	Unstandardized (b)	Standardized (β)	*P* Value	Bootstrapped 95% CI
Whole brain	−6684.22	−0.06	.38	−21717.0 to 8106.0	−6568.31	−0.06	.06	−13893.0 to 501.0
Amygdala								
Left	−34.68[b,c]	−0.18[b,c]	.007[b,c]	−60.65 to −11.01[b,c]	−4.72	−0.03	.43	−14.97 to 5.20
Right	−20.64	−0.10	.13	−45.94 to 6.65	−10.50	−0.05	.09	−23.11 to 0.75
Hippocampus								
Left	−29.51	−0.06	.30	−91.44 to 33.27	−31.83[c]	−0.07[c]	.05[c]	−61.96 to −3.03[c]
Right	14.90	0.03	.61	−43.83 to 73.13	−15.58	−0.04	.26	−43.46 to 11.25
Ventral striatum								
Left	−0.59	−0.01	.94	−15.12 to 13.15	0.26	0.00	.94	−6.09 to 7.1
Right	−20.87[b,c]	−0.22[b,c]	.005[b,c]	−35.69 to −6.44[b,c]	−2.83	−0.03	.43	−10.01 to 4.03
Orbitofrontal cortex								
Left	−88.72	−0.06	.24	−232.34 to 50.1	−26.00	−0.02	.48	−87.24 to 42.66
Right	−21.51	−0.02	.76	−156.19 to 112.22	−39.73	−0.03	.24	−106.98 to 23.96

[a] Unstandardized (b) and standardized (β) regression coefficients and their associated *P* values are presented for the effects of cannabis exposure and lifetime quantity of use (among cannabis-exposed individuals) from separate linear regression models predicting whole-brain or regional volume. Bootstrapped confidence intervals are also presented for each estimate (5000 bootstrap iterations; bias-corrected accelerated percentile intervals). Regressions controlled for sex, age (years), race/ethnicity (white vs not; African American vs not), zygosity (monozygotic vs not; dizygotic vs not), total household income, picture vocabulary, and whole-brain volume (when predicting regional volumes). Negative regression coefficients indicate smaller volumes for exposed vs unexposed individuals or greater lifetime quantity of use.

[b] Effects passing false-discovery rate (q < 0.05) correction.

[c] Effects significant at *P* < .05.

Table 3. Results of SOLAR Analyses[a]

		Amygdala		Hippocampus		Ventral Striatum		Orbitofrontal Cortex	
Variable	Whole-Brain Volume	Left	Right	Left	Right	Left	Right	Left	Right
Heritability/familiality (SE), %	95.3 (1.0)[b]	52.9 (7.8)[b]	53.6 (10.4)[b]	22.3 (9.8)[b]	77.7 (6.8)[b]	49.3 (8.2)[b]	62.4 (7.6)[b]	45.8 (8.9)[b]	60.2 (7.2)[b]
P value	<.001[b]	<.001[b]	<.001[b]	.01[b]	<.001[b]	<.001[b]	<.001[b]	<.001[b]	<.001[b]
Phenotypic correlation	−0.064	−0.175[b]	−0.091	−0.050	0.042	0.005	−0.154[b]	−0.062	−0.004
P value	>.99	.005[b]	.15	.41	.53	.94	.02[b]	.33	.95
Environmental correlation (SE)	0.062 (0.277)	0.280 (0.215)	0.059 (0.201)	0.072 (0.172)	−0.080 (0.254)	−0.026 (0.170)	−0.015 (0.197)	0.055 (0.188)	−0.275 (0.194)
P value	.82	.19	.78	.68	.76	.89	.94	.77	.17
Genetic/familial correlation (SE)	−0.090 (0.124)	−0.433 (0.139)[b]	−0.192 (0.180)	−0.220 (0.248)	0.090 (0.167)	0.027 (0.167)	−0.231 (0.148)	−0.159 (0.186)	0.145 (0.150)
P value	>.99	.004[b]	.27	.36	.55	.87	.12	.38	.32

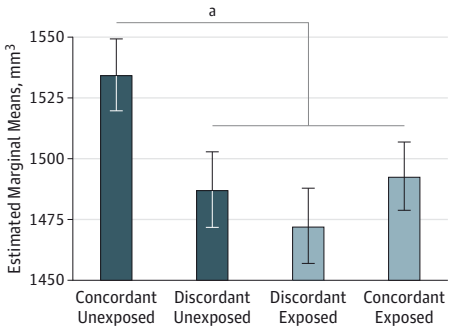
Abbreviation: SOLAR, sequential oligogenic linkage analysis routines.

[a] All models controlled for age, sex, race/ethnicity, income, picture vocabulary, and whole-brain volume (when predicting regional volumes). Heritability estimates were derived from a standard polygenic model (not including cannabis use as a covariate). Household effects (living with the same biological mother) were used to test for shared/rearing environment and found to be nonsignificant at *P* > .05. Phenotypic, environmental, and genetic/familial correlations were derived from bivariate models including each regional volume and cannabis use (ever vs never). Standard errors are presented for the estimates of heritability and environmental and genetic correlations.

[b] Effects significant at *P* < .05.

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 environmen-

Figure 2. Left Amygdala Volume by Cannabis Exposure Group



Estimated marginal means (error bars indicate standard errors) for left amygdala volumes by cannabis exposure group are presented. The significant fixed effect of contrast 3 is denoted: concordant unexposed pairs showed larger volumes than all other groups.

[a] $P < .05$.

tal 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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Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Family Structure Information and Sibling Pair Creation

173 families contributed multiple siblings to the current analysis; 11 families had data available from all four siblings, data from three siblings was available from 79 families, data from two siblings was available from 83 families, and 36 individuals were the only member of their family at the time of the June 2014 data release. This included 48 monozygotic and 45 dizygotic twin pairs where both twins had participated by this data release.

For the discordant sibling analyses, all possible pairings of siblings were drawn from the data (N=368 pairs from 173 families). For example, if all four siblings recruited from a given family had already participated by the current data release, six sibling pairs were created (sib1-sib2, sib1-sib3, sib1-sib4, sib2-sib3, sib2-sib4, sib3-sib4). Thus, any one individual could contributed to multiple paired observations. Sibling pairs were grouped as concordant exposed (both siblings ever used cannabis; N=123), concordant unexposed (both siblings never used cannabis; N=114), or discordant (one sibling reported use while their sibling never used cannabis; N=149). For ease of analysis and interpretability, the cannabis-exposed sibling in a discordant pair was always ordered first in the pair; the order of siblings was pseudo-randomized for concordant pairs to balance the sex and cannabis use distributions across siblings.

Given the confounds noted in the main text, we excluded 145 opposite-sex pairs, resulting in 241 sibling pairs (50 MZ, 45 DZ, and 146 non-twin siblings) of which 89 pairs were discordant for cannabis exposure, 81 were concordant for cannabis exposure, and 71 pairs were concordantly unexposed.

eAppendix 2. MRI Pre-Processing Information

Relevant steps from the HCP processing pipeline included: (1) Down-sampling of the 0.7mm T1w image to 1mm using splines, (2) Intensity normalization and Talairach transformation (-autorecon1), (3) Skull registration, (4) FreeSurfer skull stripping, (5) FreeSurfer subcortical segmentation (-autorecon2), and (6) Extraction of volume statistics (-segstats).

eAppendix 3. Variable Coding Information

Age of onset (1=≤14years old, 2=15-17, 3=18-20, 4=≥21)
Lifetime frequency of use (1=1-5 times used, 2=6-10, 3=11-100, 4=101-999, 5=≥1000)
Total Household Income (1=<$10,000, 2=10,000-19,999, 3=20,000-29,999, 4=30,000-39,999, 5=40,000-49,999, 6=50,000-74,999, 7=75,000-99,999, 8=>=100,000)
Alcohol use (drinks per day during the 12-month heaviest period of use 0=0, 1=1, 2=2, 3=3, 4=4, 5=5-6, 7=6+ drinks)
Non-cannabis illicit drug use (times used across the lifetime; 0=never, 1=1-2 times, 2=3-10, 3=11-25, 4= males 26-100; females ≥26, 5=males ≥100)
Childhood conduct problems (0=0, 1=1, 2=2 for males, ≥2 for females, 3=≥3 problem behaviors for males)
These variables were all available as ordinal as part of the HCP data release (none were made ordinal as part of the current analyses).
For further information see, http://www.humanconnectome.org/documentation/
https://wiki.humanconnectome.org/display/PublicData/HCP+Data+Dictionary+Public-+500+Subject+Release

eAppendix 4. Picture Vocabulary

Age-Adjusted Scale Scores from the NIH Toolbox Picture Vocabulary Test were included as a covariate in all analyses. The test was administered in a computerized adaptive format. Participants were presented with an audio recording of a word and four images on the computer screen and were asked to select the picture that most closely matched the meaning of the word. Scores are considered to be a measure of receptive vocabulary and a strong proxy of crystallized intelligence abilities. An age-adjusted score of 100 is considered average for one's age based on the NIH Toolbox normative data. Scores around 115 indicate above-average ability while individuals scoring around 130 are in the top ~2% nationally for their age. A score of 85 indicates below-average ability, while a score of 70 or below suggests significant impairment.

eAppendix 5. Additional Covariates

(a) Tobacco, alcohol, and other illicit drug use are highly comorbid with cannabis use[1], thus these were included a additional covariates to control for potential confounds between substance use and brain volumes: The SSAGA was used to assess alcohol use (drinks per day during the 12-month heaviest period of use), cigarette use (heaviness of smoking index[2], with those who had smoked <100 cigarettes lifetime coded as 0), and non-cannabis illicit drug use (times used across the lifetime).

(b) To account for increased cannabis use in individuals with certain psychopathology[3,4], which have been occasionally linked to structural variation[5,6]: Lifetime histories of DSM-IV major depressive disorder diagnosis and childhood conduct problems were also assessed by the SSAGA (see eMethods S3 for variable codings).

(c) Personality measures have been implicated as correlates of cannabis use[7] and structural variation[8]: Personality measures included neuroticism, extraversion, openness, agreeableness and conscientiousness scores (from the revised 60-item NEO five factor inventory [NEO-FFI][9] completed as part of the Penn Computerized Cognitive Battery[10,11]).

(d) Impulsivity underlies cannabis use[12] and may be an index of predisposition to onset of use and may be related to volumetric alterations: A relatively coarse measure of impulsivity was computed from the ADHD subscale of the Achenbach Adult Self-Report (ASR) for Ages 18-59[13]. Specifically, we summed responses to the items: “I am impulsive or act without thinking”, “I am too impatient”, and “I rush into things without considering the risks”. Higher sum scores indicate a higher liability to impulsive behaviors. In addition, scores on a delay discounting task were used as an additional measure of impulsivity/self-regulation. Participants made six economic decisions between a larger, delayed reward ($200) and a smaller, immediate reward to determine an ‘indifference point’ where a participant was equally likely to chose the immediate or delayed amount (for details, see [14-16]). We calculated area under the curve (AUC), a validated and reliable index of delay discounting[17]; smaller AUC indicates steeper discounting i.e. more impulsivity/less self-regulation.

eTable 1. Sibling Pairs by Cannabis Exposure, Zygosity, and Sex

Concordant Never Pairs	Sibling Pairs	DZ Pairs	MZ Pairs	Total	Mean Age Difference for Sibling Pairs (years)
Both Female	24	9	21	54	3.54
Both Male	10	4	3	17	4.40
Opposite Sex	43	0	0	43	3.60
Total	77	13	24	114	3.68
Concordant Ever Pairs	**Sibling Pairs**	**DZ Pairs**	**MZ Pairs**	**Total**	
Both Female	19	9	11	39	4.74
Both Male	28	8	6	42	3.25
Opposite Sex	42	0	0	42	3.62
Total	89	17	17	123	3.74
Discordant Pairs	**Sibling Pairs**	**DZ Pairs**	**MZ Pairs**	**Total**	
Both Female	49	13	5	67	3.45
Both Male	16	2	4	22	3.81
Female User-Male Non-User	14	0	0	14	4.64
Male User-Female Non-User	46	0	0	46	4.02
Total	125	15	9	149	3.84

A breakdown of sibling pairs (total N=386) is presented by concordance for cannabis use, pair zygosity, and sex. Pairs consisted of monozygotic twins (MZ, total N= 50 pairs), dizygotic twins (DZ, total N=45), or non-twin siblings (total N=291). Pairs were either same-sex (both female or both male) or opposite sex. All twin pairs were same-sex. The ordering of opposite sex pairs was randomized across concordant pairs, but was fixed for discordant pairs based on use. The count of pairs discordant for use and sex is presented split by female user-male non-user vs. male user-female non-user pairs. The final column presents mean age differences between individuals in non-twin sibling pairs, split by sex concordance, as well as overall mean values for each cannabis use discordance group.

eTable 2. Summary of Regression Results Controlling for Additional Covariates

	Cannabis Exposure - Ever vs. Never Used (N=483)			Lifetime Amount of Use Among Exposed Individuals (N=262)			Age of Onset Among Exposed Individuals (N=262)		
Volume (mm^3)	**b**	**t**	**p**	**b**	**t**	**p**	**b**	**t**	**p**
Whole Brain	-1807.514	-0.216	0.829	**-9438.681**	**-2.236**	**0.026**	-1646.645	-0.289	0.773
Left Amygdala	**-34.056**	**-2.369**	**0.018**	-0.512	-0.068	0.946	-0.046	-0.005	0.996
Right Amygdala	-22.143	-1.463	0.144	-8.728	-1.148	0.252	1.716	0.170	0.865
Left Hippocampus	-38.097	-1.028	0.304	**-46.840**	**-2.293**	**0.023**	24.257	0.891	0.374
Right Hippocampus	9.156	0.277	0.782	-22.543	-1.290	0.198	28.763	1.246	0.214
Left Ventral Striatum	-5.459	-0.683	0.495	-1.006	-0.233	0.816	1.986	0.349	0.728
Right Ventral Striatum	**-20.355**	**-2.428**	**0.016**	-3.910	-0.878	0.381	0.908	0.154	0.878
Left Orbitofrontal Cortex	-20.214	-0.239	0.811	9.775	0.218	0.828	-50.560	-0.855	0.393
Right Orbitofrontal Cortex	22.907	0.295	0.768	1.026	0.025	0.980	-15.913	-0.289	0.773

Unstandardized (b) regression coefficients and their associated t- and p-values for the effects of cannabis exposiure, age of onset, and lifetime quantity of use from separate linear regression models predicting whole brain or regional volume. Regressions controlled for sex, age, ethnicity (White vs. not; African American vs. not), zygosity (Monozygotic vs. not; Dizygotic vs. not), alcohol use, cigarette use, non-cannabis illicit drug use, self-reported impulsivity, NEO-FFI scores, delay discounting, major depressive disorder history, childhood conduct problems, and whole brain volume (when predicting regional volumes). Negative regression coefficients indicate smaller volumes for exposed vs. unexposed individuals, with later age of onset, or greater lifetime quantity of use. Effects significant at p<0.05 are in bold.

eTable 3. Helmert Contrast Coding

	Contrast 1 Causal Hypothesis	Contrast 2 Graded Liability Hypothesis	Contrast 3 Predispositional Hypothesis
Unexposed Individual from Discordant Pair	-1	-1	-1
Exposed Individual from Discordant Pair	1	-1	-1
Concordant Exposed Pairs	0	2	-1
Concordant Unexposed Pairs	0	0	3

The Helmert contrast coding scheme for the linear mixed model analyses is presented here. Contrast 1 compares exposed and unexposed siblings from discordant pairs. Contrast 2 compares individuals from concordant exposed pairs to individuals from discordant pairs. Contrast 3 compares individuals from concordant unexposed pairs to all other groups.

eTable 4. Relationships Between Covariates and Cannabis Use Variables

Variable	Test	Cannabis Age of Onset	Cannabis Times Used
Sex (F>M)	t-test	2.36*	-5.39***
Age (years)	Correlation	0.05	-.122*
White or Not	t-test	0.05	-2.19*
African American or Not	t-test	1.27	2.17*
Monozygotic or Not	t-test	-0.83	-0.30
Dizygotic or Not	t-test	0.55	-0.58
Total Household Income	Correlation	0.11	-0.23***
Age-Adjusted Picture Vocabulary	Correlation	0.08	-0.05
NEO Contentiousness	Correlation	0.04	-0.04
NEO Extraversion	Correlation	-0.01	-0.03
NEO Neuroticism	Correlation	0.02	-0.04
NEO Openness	Correlation	-0.07	0.28**
NEO Agreeableness	Correlation	0.16**	-.017**
Delay Discounting	Correlation	-0.04	0.09
Impulsivity	Correlation	-0.11	0.09
Alcohol Use	Correlation	-0.22**	0.30**
Cigarette Use	Correlation	-0.35**	0.36**
Illicit Drug Use	Correlation	-.034**	0.53**
Depression History	t-test	-1.20	2.03*
Childhood Conduct Problems	Correlation	-0.20**	0.24**

Ordinal variables for age of onset of cannabis use and lifetime times using cannabis were related to all covariates of interest by either t-test (for binary covariates) or correlation. Thus, values represent t-statistics for t-test results and Pearson's r for correlations. Note that earlier age of onset relates to more lifetime use ($r(260)= -0.42$, $p<0.001$), so observed relationships with covariates tend to be in opposite directions for these two variables, i.e. females begin using cannabis at a later age and use less over their lifetime.
** $p<0.05$, ** $p<0.01$, *** $p<0.001$*

eTable 5. Descriptive Statistics and Intercorrelations Among Brain Volumes

			Inter-Correlations							
	Mean	SD	Left Amyg	Right Amyg	Left HC	Right HC	Left VS	Right VS	Left OFC	Right OFC
Whole Brain Volume	1116835.18	112422.14	0.71	0.72	0.67	0.71	0.55	0.57	0.83	0.84
Left Amygdala (Amyg)	1526.22	192.22		0.81	0.68	0.67	0.42	0.44	0.57	0.60
Right Amygdala (Amyg)	1610.18	204.61			0.65	0.66	0.42	0.41	0.57	0.60
Left Hippocampus (HC)	4344.16	465.88				0.78	0.39	0.34	0.46	0.53
Right Hippocampus (HC)	4406.23	439.93					0.44	0.44	0.48	0.57
Left Ventral Striatum (VS)	557.04	92.45						0.70	0.53	0.55
Right Ventral Striatum (VS)	597.09	98.02							0.57	0.59
Left Orbitofrontal Cortex (OFC)	12369.45	1417.05								0.82
Right Orbitofrontal Cortex (OFC)	12233.55	1339.60								

Mean and standard deviation (SD) values are presented for each brain volume of interest (N=483). Pearson's correlation between all volumes of interest were also presented. All correlations were significant p<0.001.

eTable 6. Interrelationships Among Covariates

	Age	White	African Am.	MZ Twin	DZ Twin	NEO-C	NEO-E	NEO-N	NEO-O	NEO-A	Delay Disc.	Impuls.	Alcohol	Cig.	Illicit	MDD	Conduct
Sex (F>M)	0.63	0.06	0.06	13.81***	1.54	2.52*	0.37	3.02**	-2.21*	3.32**	-1.01	-2.54*	-2.64**	-2.58*	-4.20***	0.00	-0.29**
Age (years)		2.03*	-1.70	3.33**	2.97**	0.07	0.00	-0.03	-0.12*	0.11*	0.03	-0.15**	0.02	0.11*	0.02	-0.64	0.03
White or Not			-	11.55**	0.72	-1.12	1.60	0.29	-1.11	3.93***	7.09***	-1.12	3.43**	-0.49	0.77	2.81	-0.12**
African Am. or Not				7.02*	0.33	2.02*	-1.05	-1.09	0.57	-2.89**	-7.31***	0.44	-3.83***	0.92	-1.92	1.43	0.09
Monozygotic or Not					-	4.65***	3.86***	-2.37*	-2.38*	4.78***	0.42	-4.18***	0.63	-0.07	-0.85	2.41	-0.10*
Dizygotic or Not						-1.42	-1.05	0.14	-1.55	0.81	-1.23	-2.22*	-0.60	1.24	0.34	0.63	-0.07
NEO Contentiousness							0.35**	-0.42**	-0.06	0.24**	-0.04	-0.26**	0.03	-0.02	-0.11*	-2.26*	-0.03
NEO Extraversion								-.379**	0.06	0.34**	-0.01	-0.02	0.14**	-0.01	0.01	-4.49***	-0.03
NEO Neuroticism									0.01	-0.29**	-0.03	0.24**	-0.06	-0.01	-0.01	5.20***	0.07
NEO Openness										0.13**	0.08	0.00	0.05	0.03	0.19**	2.70**	0.11*
NEO Agreeableness											.090*	-.031**	-0.11*	-0.17**	-0.10*	-2.20*	-0.27**
Delay Discounting												-0.08	-0.07	-0.13**	0.03	1.79	0.00
Impulsivity													0.06	0.01*	0.02	1.94	0.12**
Alcohol Use														0.28**	0.31**	-0.31	0.14**
Cigarette Use															0.36**	0.61	0.11*
Illicit Drug Use																0.89	0.27**
Depression History																	0.21**

Values represent t-statistics when comparing a binary and a continuous variable by independent-samples t-test, chi^2 statistics when comparing two binary variables, or Pearson's r coefficients relating two continuous variables. Sex, ethnicity, zygosity, and depression history are binary variables.
** p<0.05, ** p<0.01, *** p<0.001*

eTable 7. Full Cannabis Exposure Regression Results

	WBV	L. Amyg.	R. Amyg.	L. Hipp.	R. Hipp.	L. Accumb.	R. Accumb.	L. OFC	R. OFC
Intercept	**1114080.197*** **	**523.688*** **	**405.275** **	**1426.197*** **	**1540.894*** **	50.159	**163.683* **	**1555.591* **	**1918.633** **
Cannabis Use	-6684.224	**-34.676** **	-20.644	-29.514	14.900	-0.585	**-20.866** **	-88.716	-21.509
Whole Brain Volume	-	**0.001*** **	**0.001*** **	**0.002*** **	**0.002*** **	**0.001*** **	**0.001*** **	**0.01*** **	**0.01*** **
Sex (F>M)	**-145402.729*** **	**-71.587*** **	**-51.932** **	-45.333	-62.71	10.119	-3.252	-46.98	21.655
White or Not	**39478.07* **	-4.96	13.682	138.584	**252.801*** **	-27.226	-11.341	-55.404	-13.105
African American or Not	-2721.389	-11.602	-1.853	65.114	**220.602** **	-1.623	8.176	-108.448	-86.000
Age (years)	-1684.557	-1.357	-0.442	1.997	0.798	-2.031.	**-3.129** **	-16.84	**-34.557** **
Monozygotic or Not	-9596.319	-3.394	-11.872	**-113.456** **	-47.686	-9.438	-10.208	-174.268.	-144.654
Dizygotic or Not	3431.441	17.936	16.554	-61.935	-56.675	**-18.192* **	-3.469	-69.531	-69.696
Income	**6715.404*** **	3.38	2.41	-2.55	-2.55	13.368	-1.80	-1.75	29.65
Picture Vocabulary	**937.851*** **	-0.44	-0.41	1.62	1.62	1.22	0.38	0.00	0.08

Unstandardized regression coefficients for cannabis exposure (ever vs. never used cannabis) and all covariates predicting whole brain volume and left and right amygdala, hippocampus, ventral striatum, and orbitofrontal cortex volumes are presented. Significant effects are in bold.
** p<0.05, ** p<0.01, *** p<0.001*

eTable 8. Full Times Used Regression Results

	WBV	L. Amyg.	R. Amyg.	L. Hipp.	R. Hipp.	L. Accumb.	R. Accumb.	L. OFC	R. OFC
Intercept	**1148775.959*** **	**487.155** **	**487.577** **	**2035.91*** **	**2147.548*** **	126.569	**220.057***	1724.505	**3044.788*** **
Cannabis Use	-6568.307	-4.723	-10.497	-31.834	-15.578	0.256	-2.834	-26.001	-39.727
Whole Brain Volume	-	**0.001*** **	**0.001*** **	**0.002*** **	**0.002*** **	**0.001*** **	**0.001*** **	**0.010*** **	**0.009*** **
Sex (F>M)	**-162659.104*** **	**-71.631** **	**-67.823** **	-78.05	-94.895	3.584	-18.274	-88.973	-211.221
White or Not	32149.864	-13.079	-5.416	103.305	154.803.	-21.465	-3.088	-40.713	67.811
African American or Not	242.661	-16.501	-16.268	11.313	52.563	1.856	12.643	-165.457	29.456
Age (years)	-2452.965	0.308	-0.261	-9.220	-6.608	-2.129	-2.825	-27.889	**-32.023***
Monozygotic or Not	-4965.48	-34.71	-29.256	-92.885	-57.716	-9.393	-12.469	-192.975	**-276.029***
Dizygotic or Not	1123.352	-8.526	-9.297	-83.447	-67.827	-11.714	-2.038	-128.261	-208.752.
Income	**6034.039***	7.27	1.72	14.93	14.93	**32.772** **	-2.03	-1.55	29.66
Picture Vocabulary	**1054.727** **	-1.053.	-0.56	-0.92	-0.92	-0.30	-0.09	-0.12	3.64

Unstandardized regression coefficients for times using cannabis among exposed individuas (N=262) and all covariates predicting whole brain volume and left and right amygdala, hippocampus, ventral striatum, and orbitofrontal cortex volumes are presented. Significant effects are in bold.
** p<0.05, ** p<0.01, *** p<0.001*

eTable 9. Full Age at Onset Regression Results

	WBV	L. Amyg.	R. Amyg.	L. Hipp.	R. Hipp.	L. Accumb.	R. Accumb.	L. OFC	R. OFC
Intercept	**1119521.438*** **	**453.069** **	**411.452***	**1799.812*** **	**2012.669*** **	126.42	**198.645***	1566.643	**2753.595** **
Cannabis Use	721.671	2.347	5.578	21.933	29.314	1.792	2.33	-15.677	24.066
Whole Brain Volume	-	**0.001*** **	**0.001*** **	**0.003*** **	**0.002*** **	**0.001*** **	**0.001*** **	**0.010*** **	**0.009*** **
Sex (F>M)	**-155918.707*** **	**-65.874** **	**-55.155***	-41.383	-83.441	2.601	-15.141	-47.293	-164.308
White or Not	32644.176	-13.826	-7.229	95.681	143.199	-22.237	-3.926	-32.708	59.696
African American or Not	-1758.147	-19.233	-22.599	-11.475	28.134	0.632	10.345	-160.066	3.381
Age (years)	-2343.358	0.391	-0.08	-8.724	-6.559	-2.153	-2.785	-27.134.	**-31.369***
Monozygotic or Not	-6841.627	-35.534	-30.996	-96.912	-55.055	-8.869	-12.734	-204.637	**-281.879***
Dizygotic or Not	580.846	-8.853	-10.01	-85.421	-68.094	-11.624	-2.2	-131.135	-211.34
Income	**6936.764***	7.747	2.76	17.79	17.79	**33.118** **	-2.16	-1.32	33.90
Picture Vocabulary	**1047.632** **	-1.083	-0.63	-1.16	-1.16	-0.56	-0.11	-0.14	3.69

Unstandardized regression coefficients for cannabis age of onset among exposed individuals (N=262) and all covariates predicting whole brain volume and left and right amygdala, hippocampus, ventral striatum, and orbitofrontal cortex volumes are presented. Significant effects are in bold.
** p<0.05, ** p<0.01, *** p<0.001*

eTable 10. Volume Relationships With Cannabis Use by Light vs Heavier Use

	Left Amygdala			Right Ventral Striatum		
	Robust Coefficient	**t**	**p**	**Robust Coefficient**	**t**	**p**
Intercept	**533.252**	**3.71**	**<0.001**	**170.499**	**2.38**	**0.018**
Cannabis Use <100 times	**-32.479**	**-2.37**	**0.019**	**-19.549**	**-2.30**	**0.023**
Cannabis Use ≥100 times	**-43.323**	**-2.25**	**0.026**	**-26.663**	**-2.25**	**0.025**
Whole Brain Volume	**0.001**	**9.25**	**<0.001**	**.0004**	**10.94**	**<0.001**
Sex (F>M)	**-74.784**	**-3.47**	**0.001**	-5.629	-0.52	0.603
White or Not	-5.592	-0.21	0.832	-11.907	-0.69	0.489
African American or Not	-12.950	-0.45	0.657	6.916	0.36	0.721
Age (years)	-1.472	-0.74	0.460	**-3.218**	**-2.42**	**0.017**
Monozygotic or Not	-2.062	-0.12	0.908	-9.192	-0.85	0.395
Dizygotic or Not	18.900	1.25	0.214	-2.675	-0.26	0.792
Income	3.350	1.02	0.307	-1.714	-0.77	0.444
Picture Vocabulary	-0.410	-0.75	0.454	0.023	0.07	0.943
Test comparing coefficients for cannabis use <100 vs. ≥100 times	F(1, 204)=0.32, p=0.574			F(1, 204)=0.35, p=0.553		

Analyses conducted in STATA with dummy coded variables representing lifetime cannabis use of <100 and ≥100 times, with never use as the reference group. Individual estimates indicate that both dummy codes (<100 and ≥100 times) are significantly associated with brain volumes. Post-hoc comparisons between the estimates for each dummy tested whether the difference between the coefficients for <100 and ≥100 times could be equated to zero (i.e. not statistically different from each other). A non-significant result indicates that there are no significant differences in the magnitude of association between brain volumes and using cannabis <100 and ≥100 times. A robust sandwich variance estimator was used to adjust standard errors for familial clustering.

eTable 11. Linear Mixed Model Results for Same-Sex Sibling Pairs

Effect	WBV	L. Amyg	R. Amyg	L. HC	R. HC	L. VS	R. VS	L. OFC	R. OFC
Intercept	**1055403.1*** **	**338.80** **	**140.70**	**872.60** **	**895.00*** **	78.020	**169.30***	93.330	**2333.00*** **
Whole Brain Volume	-	**0.001*** **	**0.001*** **	**0.003*** **	**0.003*** **	**0.001*** **	**0.001*** **	**0.011*** **	**0.01*** **
Sex (F>M)	-142727***	-43.98#	-5.431	-0.151	-3.560	16.720	5.454	173.000	44.490
White or Not	17771.10	37.350	14.390	117.700	125.900	**-47.07***	-6.592	-126.200	-27.990
African American or Not	-11327.00	39.860	-13.710	73.310	139.700	-21.000	11.850	-32.470	-83.420
Age (years)	188.800	-0.496	-0.139	5.345	2.868	-2.082#	**-3.011***	-15.490	**-48.78*** **
Monozygotic vs. Sibling Pair	-918.800	-14.990	-14.400	**-87.49***	-7.614	-5.892	-14.580	-22.470	-28.920
Dizygotic vs. Sibling Pair	2714.000	7.459	15.390	-40.200	-35.670	-12.690	-6.443	85.380	-12.800
Income	**4799.9** **	1.779	-2.542	-6.074	0.951	-1.479	**-4.572***	-15.140	-13.730
Picture Vocabulary	**970.5*** **	0.691	0.388	**4.373*** **	**4.914*** **	-0.225	-0.399	1.090	-0.808
Contrast 1	-6274.600	-7.431	-1.961	16.430	23.810	-1.221	-1.628	59.520	-30.180
Contrast 2	-3523.900	4.312	-0.902	1.098	4.899	-1.785	-2.151	-12.600	-10.380
Contrast 3	1803.400	**12.56** **	7.743#	6.028	-9.214	-2.088	2.731	8.593	4.190

*Estimates of fixed effects from the linear mixed model analyses examining same-sex sibling pairs are presented. Effects significant at p<0.05 are in bold; significant effects of the Helmert contrasts (eTable 2) are shaded gray. #p<0.10, *p<0.05, **p<0.01, ***p<0.001*

eTable 12. Linear Mixed Model Results for All Sibling Pairs

Effect	WBV	L. Amyg	R. Amyg	L. HC	R. HC	L. VS	R. VS	L. OFC	R. OFC
Intercept	**1067574.5*** **	**386.7*** **	184.50#	**803.10** **	**1049.00*** **	96.57#	**159.00** **	**1135.00***	**1607.00*** **
Whole Brain Volume	-	**0.001*** **	**0.001*** **	**0.003*** **	**0.003*** **	**0.001*** **	**0.001*** **	**0.011*** **	**0.01*** **
Sex (F>M)	**-144400.3*** **	**-66.01*** **	-23.940	-33.350	-22.730	5.597	-0.770	33.540	24.190
White or Not	25077.000	32.560	-12.320	98.530	**233.9*** **	**-41.03***	-9.898	-208.200	19.730
African American or Not	-8892.600	31.870	-25.930	83.030	**239** **	-15.070	8.002	-261.000	-103.300
Age (years)	-791.700	1.110	1.003	6.062	2.494	**-1.928***	**-2.957** **	-17.97#	**-29.68*** **
Monozygotic vs. Sibling Pair	-2823.200	-6.607	-6.625	-72.07#	1.103	-1.335	-14.45#	-30.890	-62.370
Dizygotic vs. Sibling Pair	1026.700	11.490	23.890	-34.360	-45.630	-10.670	-6.308	78.370	-34.870
Income	**4836.3*** **	2.815	-1.324	-2.121	8.665	-2.441#	**-3.082***	-12.320	-22.43#
Picture Vocabulary	**1237*** **	0.454	0.534	**3.789*** **	**3.003** **	-0.093	-0.312	-0.488	1.959
Sex Concordant vs. Discordant Pair	-9268.400	-7.066	-9.236	29.120	5.840	-1.617	4.582	-101.700	-71.410
Contrast 1	8125.200	**-25.88** **	-13.440	-18.530	-25.090	-5.044	**-16.2** **	0.867	-39.630
Contrast 2	-2892.900	0.103	-0.294	-5.580	4.891	-3.060	-4.179#	-26.590	-10.590
Contrast 3	1665.000	**9.511** **	5.800	7.149	-3.367	-0.767	3.414#	-2.388	7.950
Contrast 1 x Sex Concordance	**-14930.5***	19.280	11.830	37.120	50.81#	3.517	**14.91***	53.590	17.340

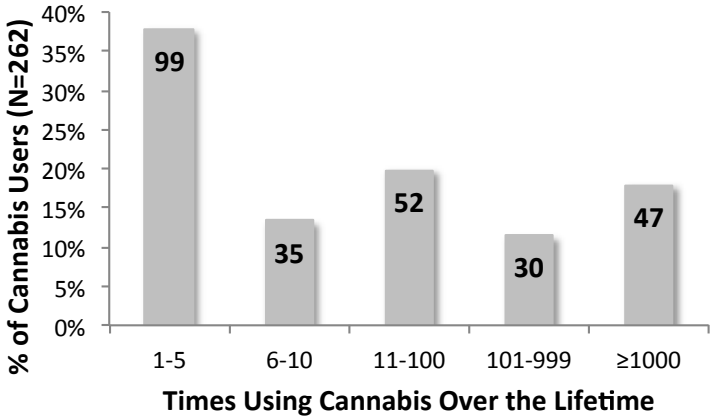
*Estimates of fixed effects from the linear mixed model analyses examining all sibling pairs are presented. Effects significant at p<0.05 are in bold; significant effects of the Helmert contrasts (eTable 2) are shaded gray. #p<0.10, *p<0.05, **p<0.01, ***p<0.001*

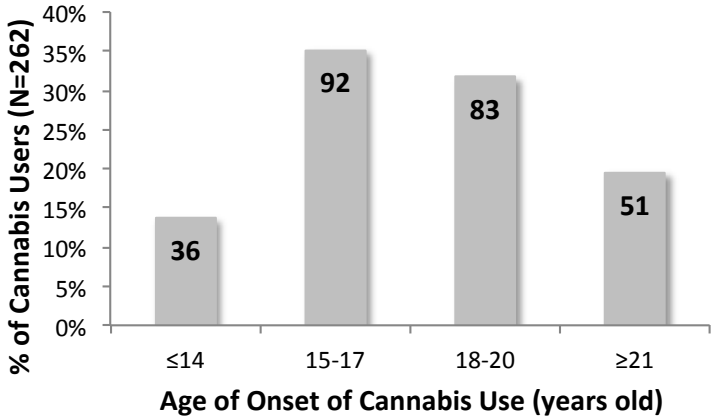
eTable 13. Control Analysis Results

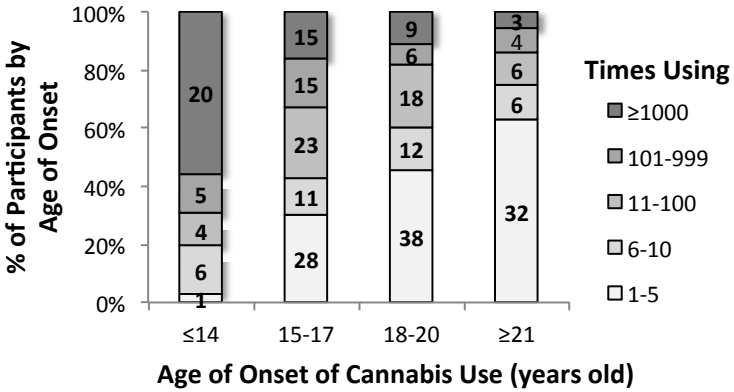
	Discordant Users vs. Unrelated Individuals	
	t	**p-value**
Whole Brain Volume	-1.69	0.09
Left Amygdala	**-3.44**	**0.001**
Right Amygdala	-1.69	0.09
Left Hippocampus	-0.99	0.33
Right Hippocampus	-0.41	0.68
Left Ventral Striatum	0.34	0.74
Right Ventral Striatum	-1.24	0.22
Left Orbitofrontal Cortex	-1.72	0.09
Right Orbitofrontal Cortex	-1.98	0.05

We compared the cannabis-exposed individuals from same-sex discordant pairs (N=89) with unrelated *but sex-matched unexposed individuals using a pair t-test. T-statistics and their associated p-value for the paired t-test are presented.*

eFigure. Histogram of Age at Onset and Times Using Cannabis







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