Archival Report

Diminished Structural Brain Integrity in Long-term Cannabis Users Reflects a History of Polysubstance Use

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ABSTRACT

BACKGROUND: Cannabis legalization and use are outpacing our understanding of its long-term effects on brain and behavior, which is fundamental for effective policy and health practices. Existing studies are limited by small samples, cross-sectional measures, failure to separate long-term from recreational use, and inadequate control for other substance use. Here, we address these limitations by determining the structural brain integrity of long-term cannabis users in the Dunedin Study, a longitudinal investigation of a population-representative birth cohort followed to midlife.

METHODS: We leveraged prospective measures of cannabis, alcohol, tobacco, and other illicit drug use in addition to structural neuroimaging in 875 study members at age 45 to test for differences in both global and regional gray and white matter integrity between long-term cannabis users and lifelong nonusers. We additionally tested for dose-response associations between continuous measures of cannabis use and brain structure, including careful adjustments for use of other substances.

RESULTS: Long-term cannabis users had a thinner cortex, smaller subcortical gray matter volumes, and higher machine learning–predicted brain age than nonusers. However, these differences in structural brain integrity were explained by the propensity of long-term cannabis users to engage in polysubstance use, especially with alcohol and tobacco.

CONCLUSIONS: These findings suggest that diminished midlife structural brain integrity in long-term cannabis users reflects a broader pattern of polysubstance use, underlining the importance of understanding comorbid substance use in efforts to curb the negative effects of cannabis on brain and behavior as well as establish more effective policy and health practices.

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Increasing legalization of cannabis has been accompanied by a decrease in perceived risks of use (1), despite emerging evidence of numerous adverse outcomes in long-term users, including impaired cognitive function (2–7). Neuroimaging studies of cannabis users have further revealed structural alterations in both gray and white matter, supporting the very cognitive functions found to be impaired in chronic users (8–10). Most studies of structural brain integrity in cannabis users have reported on gray matter, with the most consistent differences found in cannabinoid receptor–dense regions including the hippocampus and amygdala (1,9,10). Specifically, meta-analyses have found smaller hippocampal volumes in cannabis users (11,12), with findings in other regions being more mixed. Results from white matter studies are also mixed, with evidence for associations between earlier onset of use and reduced white matter microstructural integrity most consistently emerging (8,13). However, several studies have

found no evidence of gray or white matter alterations in cannabis users (13–17), and additional studies have suggested that findings may be attributable to confounding influences of polysubstance use (18) or predispositional factors (19).

The absence of clear evidence for structural brain alterations in long-term cannabis users reflects, in part, limitations of existing studies that must be addressed to better understand the nature of adverse outcomes associated with long-term drug use and inform public health policy and practice. First, as with the broader clinical neuroimaging literature, heterogeneous study designs and small unrepresentative samples limit power to detect associations beyond a few regions of interest or generalize to other populations (18). In addition, existing studies face limitations common to the study of cannabis users, namely, accurate usage quantification and reliance on cross-sectional retrospective usage reports (20). Reported usage levels vary widely across studies, from as little

as 1 to >1000 lifetime uses (11,13). Further, cannabis users often engage in polysubstance use, with 1 population-based study showing that rates of alcohol, tobacco, and other illicit drug use among cannabis users were at least 2, 4, and 5 times that of nonusers, respectively (21). As such, disentangling effects of cannabis from other substances is challenging, and more research is needed to determine how the brains of cannabis users compare with those of other substance users (4). Finally, existing findings are largely based on studies of adolescents and young adults (22), some of whom used cannabis very infrequently (11), leaving open questions about the structural brain integrity of long-term cannabis users in midlife or older adulthood. As the number of older adults using cannabis reaches historical highs (23), it is increasingly important to identify how long-term use contributes to diminished cognitive reserves (24,25) and increased risk for aging-related diseases such as Alzheimer's disease and related dementias (ADRDs) (26,27).

We sought to fill these gaps by evaluating the midlife structural brain integrity of long-term cannabis users in the Dunedin Study, which offers a rich longitudinal dataset of health and behavioral measures in a population-representative birth cohort of 1000 people followed to age 45 years, when neuroimaging data were first collected. To our knowledge, this neuroimaging dataset is among the largest of long-term cannabis users and the only large dataset with detailed prospective substance use measures. With this dataset, we leveraged repeated prospective cannabis use assessments over nearly 30 years to compare long-term cannabis users to lifelong nonusers and to test whether associations with brain structure depend on usage level (i.e., are dose dependent). Detailed prospective alcohol and tobacco use measures allowed us to test whether structural brain alterations in long-term cannabis users are specific to their cannabis use or are potentially explained by the fact that they also use other substances heavily. In addition, these measures allowed us to compare the magnitude of alterations in alcohol and tobacco users to those of cannabis users. Finally, the availability of high-quality brain structure measures at age 45 in a large number of study members with documented drug use histories (n = 860 for gray matter; n = 853 for white matter) afforded statistical power to conduct unbiased exploratory whole-brain analyses to ascertain the breadth of associations between long-term cannabis use and midlife structural brain integrity.

Specifically, we evaluated long-term cannabis users on comprehensive measures of global and regional gray matter (i.e., cortical thickness [CT], surface area [SA], subcortical volume) and white matter (i.e., microstructural integrity as indexed by fractional anisotropy [FA], and white matter hyperintensity [WMH] volume) in midlife. These magnetic resonance imaging measures not only have shown alterations in regular cannabis users (1) but also represent promising midlife biomarkers of accelerated cognitive decline and risk for ADRDs (28). In addition to gray and white matter, we examined links with brain age, a machine learning–based estimate derived from multiple magnetic resonance imaging measures (29). The difference between brain age and chronological age offers an approximation of age-related deterioration in the brain using cross-sectional data (30). Individuals with the same chronological age but older brain age have accelerated

cognitive decline (29), increased risk for ADRDs (31), and higher mortality (32). Collectively, these design features presented a unique opportunity to establish a comprehensive portrait of midlife structural brain integrity in long-term cannabis users, which may be important in shaping their trajectories of healthy and unhealthy aging.

METHODS AND MATERIALS

A brief description of the samples and measures is reported below. A full description is provided in Supplemental Methods.

Study Design and Participants

Participants were members of the Dunedin Study, a population-representative birth cohort (N = 1037) born between April 1972 and March 1973 in Dunedin, New Zealand. Assessments were conducted at birth and every few years, most recently at age 45, when neuroimaging was additionally conducted in 875 study members. The relevant ethics committees approved the study, and all study members provided written informed consent.

Long-term Cannabis Users and 3 Comparison Groups

At each of the 6 adult study waves (ages 18, 21, 26, 32, 38, and 45), study members self-reported the number of days (0–365) they used cannabis, the number of tobacco cigarettes smoked per day, and the number of days they used alcohol in the past year. At the 4 study waves from ages 26 to 45, study members additionally reported the number of days they used other drugs in the past year. This was used to assess past-year drug dependencies and identify long-term cannabis users and 3 comparison groups. Long-term cannabis users (n = 82; 65% men) used cannabis at least weekly in the past year at age 45 or were dependent on cannabis at age 45, and also used at least weekly during 1 or more previous waves. Lifelong cannabis nonusers (n = 192; 41% men) never used cannabis, had no substance use disorder diagnoses at any assessment, and never used tobacco daily. Long-term tobacco users (n = 70; 40% men) smoked tobacco daily in the past year at age 45 and at 1 or more previous waves. Long-term alcohol users (n = 56; 55% men) were at least past-year weekly drinkers at age 45 and had an alcohol dependence diagnosis at 2 or more waves. Long-term tobacco and alcohol users were mostly free from cannabis use at age 45 and had no history of cannabis dependence or weekly use. All comparison groups are mutually exclusive to long-term cannabis users but not to each other (Figure S1).

Persistence of Cannabis Use

We created two continuous cannabis use measures: persistence of cannabis dependence and persistence of regular cannabis use. Persistence of cannabis dependence comprised 1) study members who never used cannabis (n = 248); 2) study members who used but were never diagnosed (n = 468); and those who were diagnosed at 3) one wave (n = 78), 4) two waves (n = 34), 5) three waves (n = 30), and 6) four or more waves (n = 16). Persistence of regular cannabis use (≥4 times/week) comprised 1) study members who never used cannabis

(n = 248); 2) study members who used cannabis but never regularly (n = 486); and those who used regularly at 3) one wave (n = 49), 4) two waves (n = 31), 5) three waves (n = 31), or 4) four or more waves (n = 29).

Persistence of Alcohol and Tobacco Use

Continuous alcohol and tobacco dependence measures mirrored the cannabis measure. Persistence of alcohol/tobacco dependence comprised 1) study members who never used (n = 47/427); 2) study members who used but were never diagnosed (n = 504/123); and those who were diagnosed at 3) one wave (n = 167/99), 4) two waves (n = 79/81), 5) three waves (n = 45/59), or 4) four or more waves (n = 30/85).

Midlife Brain Structure

Gray matter integrity was estimated using CT and cortical SA measures extracted from the whole brain and the 360 regions in the HCP-MPP1.0 atlas (33). In addition, total brain volume (TBV) and gray matter volumes (GMVs) for 10 subcortical structures were extracted using FreeSurfer's (https://surfer.nmr.mgh.harvard.edu/) aseg parcellation.

White matter integrity was estimated using FA measures averaged across the full white matter skeleton and within tractwise regions of interest from the intersection of the skeleton and the 27 regions in the Johns Hopkins University white matter atlas (34).

Total WMH volume was calculated using UBO detector (35).

Finally, the brain age gap estimate, or the difference between chronological age predicted from magnetic resonance imaging measures of brain structure and actual chronological age (brainAGE), was estimated using a pretrained publicly available algorithm (29).

Covariates

Long-term cannabis users often regularly use other licit and illicit substances (36). Thus, we included covariates for persistent use of other substances in our models. Persistent alcohol and tobacco dependence were measured as described above. In addition, persistent illicit drug use was defined as a diagnosis of dependence on illicit drugs (other than cannabis) at 2 or more waves from ages 26 to 45 (Supplemental Methods).

Statistical Analyses

To test whether long-term cannabis users exhibit global alterations in midlife brain structure, we used t tests comparing long-term cannabis users to each comparison group on 6 sex-adjusted global brain structure measures: average CT, total SA, TBV, average FA, WMH volume, and brainAGE. To test for dose-response associations, we used ordinary least squares regression with each of the 2 continuous cannabis use measures. To compare the effect sizes of the associations between persistence of cannabis use and brain structure and those of persistence of alcohol and tobacco use, we used ordinary least squares regression with these respective measures. All dose-response analyses were adjusted for sex and subsequently additionally adjusted for persistent dependence on other substances (cannabis, alcohol, tobacco, and illicit drugs, as appropriate). For each model, we corrected for multiple comparisons across the 6 tests performed for each brain measure

using a false discovery rate (FDR) procedure (37). We report standardized beta coefficients.

We further probed regional subdivisions of 4 of the 6 global measures to examine whether associations with global measures may be driven by localized patterns of structural differences. Specifically, we conducted all group comparisons and dose-response analyses with CT and SA in the 360 regions comprising the HCP-MPP1.0 atlas (33), GMVs in the 10 subcortical volumes, and FA in the 27 tracts as described above. Given the exploratory nature of our analyses, only results with FDR-corrected p (p_{FDR}) < .05 were considered statistically significant. Analyses were conducted in R version 3.6.0 (38), preregistered (https://sites.duke.edu/moffittcaspiprojects/files/2021/07/Knodt_2020a.pdf), and checked for reproducibility by an independent analyst.

Secondary Analyses

We conducted additional preregistered analyses comparing long-term cannabis users to midlife recreational cannabis users and cannabis quitters, as well as dose-response analyses adjusting for childhood risk factors to assess any role that antecedents to substance misuse might have in our results (Supplemental Methods). At the request of a reviewer, we additionally repeated the global dose-response analyses for each sex separately.

RESULTS

Cohort Characteristics

Table 1 shows substance use patterns for the 875 study members who underwent neuroimaging at age 45, along with basic demographic information and childhood risk factors for substance misuse. While the long-term alcohol and tobacco groups were free from regular cannabis use and cannabis dependence by design, long-term cannabis users had an elevated incidence of dependence on tobacco (23.5%), alcohol (20.7%), and other illicit drugs (15.9%) at age 45.

Global Brain Structure in Long-term Cannabis Users

Group comparisons revealed that compared with lifelong cannabis nonusers, study members who were long-term cannabis users had significantly thinner average cortex (p_{FDR} = .007) and older brainAGE (p_{FDR} < .001) (Table 2; Table S1). Long-term cannabis users did not differ from nonusers on cortical SA, TBV, average FA, or WMH volume. Long-term cannabis users did not differ from alcohol or tobacco users on the 6 global brain structure measures.

Tests of dose-response associations using the continuous measure of persistence of cannabis dependence again revealed significant associations with 2 of the 6 measures: study members with more persistent dependence had thinner average cortex (p_{FDR} = .007) and older brainAGE (p_{FDR} = .010) than those with less persistent dependence (Figure 1; Table S2). Persistence of regular cannabis use showed significant associations with 5 of the 6 measures: study members who used more persistently had thinner average cortex (p_{FDR} = .002) and older brainAGE (p_{FDR} = .001), along with smaller total cortical SA (p_{FDR} = .024), smaller TBV (p_{FDR} = .002), and lower

Table 1. Sociodemographic Characteristics and Substance Use Involvement for the Full Imaging Cohort as Well as Long-term Cannabis Users and Three Comparison Groups Within the Imaging Cohort

Characteristics	Imaging Cohort, $n = 875$	Long-term Cannabis Users, $n = 82$	Lifelong Cannabis Nonusers, $n = 192$	Long-term Tobacco Users, $n = 70$	Long-term Alcohol Users, $n = 56$
Sociodemographics					
Male sex	441 (50.4%)	53 (64.6%)	79 (41.1%)	28 (40.0%)	31 (55.4%)
Childhood SES	3.76 (1.13)	3.40 (1.07)	3.92 (1.17)	3.18 (0.96)	3.79 (1.19)
Childhood self-control problems	−0.03 (0.95)	0.36 (1.09)	−0.19 (0.88)	0.44 (1.18)	0.00 (0.92)
Childhood IQ	100.7 (13.9)	98.6 (13.6)	101.4 (13.8)	92.1 (14.0)	98.1 (11.3)
Family history of substance dependence	0.15 (0.17)	0.20 (0.20)	0.10 (0.13)	0.19 (0.18)	0.14 (0.15)
Age 45 depression diagnosis	140 (16.0%)	22 (26.8%)	16 (8.3%)	10 (14.3%)	17 (30.4%)
Age 45 anxiety disorder diagnosis	165 (18.9%)	18 (22.0%)	30 (15.6%)	25 (35.7%)	16 (28.6%)
Substance Use at Age 45					
Cannabis use frequency[a]	25.8 (82.7)	255.6 (116.7)	0 (0)	0.11 (0.5)	0.32 (1.2)
Weekly cannabis use	85 (9.7%)	81 (98.8%)	0 (0%)	0 (0%)	0 (0%)
Regular cannabis use[b]	53 (6.1%)	52 (63.4%)	0 (0%)	0 (0%)	0 (0%)
Daily tobacco use	186 (21.3%)	53 (64.6%)	0 (0%)	70 (100%)	10 (17.9%)
Weekly alcohol use	814 (93.1%)	75 (91.5%)	175 (91.1%)	63 (90%)	56 (100%)
Cannabis dependence	19 (2.2%)	19 (23.5%)	0 (0%)	0 (0%)	0 (0%)
Tobacco dependence	103 (11.8%)	37 (45.1%)	0 (0%)	36 (52.2%)	6 (10.7%)
Alcohol dependence	100 (11.4%)	17 (20.7%)	0 (0%)	6 (8.6%)	30 (53.6%)
Illicit drug dependence	28 (3.2%)	13 (15.9%)	0 (0%)	1 (1.4%)	1 (1.8%)

Childhood variables are described in the Supplemental Methods. Values are presented in n (%) or mean (SD).

SES, socioeconomic status.

[a]Number of days used in past year.

[b]Regular use = 4+ days/wk.

average white matter FA ($p_{FDR} = .024$) than those who used less persistently (Figure 1; Table S2). However, no associations survived adjustment for persistent dependence on other substances (all $p_{FDR} > .2$).

Global Brain Structure in Long-term Alcohol and Tobacco Users

Persistence of alcohol dependence showed significant associations with 4 of the 6 measures: study members with more persistent alcohol dependence had thinner average cortex ($p_{FDR} < .001$), smaller TBV ($p_{FDR} = .015$), lower average white matter FA ($p_{FDR} = .003$), and older brainAGE ($p_{FDR} < .001$) than those with less persistent dependence (Figure 1; Table S3). Again, however, no associations survived adjustment for persistent dependence on other substances (all $p_{FDR} > .07$).

Persistence of tobacco dependence showed significant associations with 5 of the 6 measures: study members with more persistent tobacco dependence had thinner average cortex ($p_{FDR} < .001$), smaller total cortical SA ($p_{FDR} = .007$), smaller TBV ($p_{FDR} = .007$), lower average white matter FA ($p_{FDR} = .006$), and older brainAGE ($p_{FDR} < .001$) than those with less persistent dependence (Figure 1; Table S3). The associations between tobacco dependence and CT, SA, and brainAGE survived adjustment for other substances (all $p_{FDR} < .03$).

Regional Brain Structure in Long-term Cannabis Users

Group comparisons revealed that study members who were long-term cannabis users had significantly thinner cortex than lifelong cannabis nonusers in 34 of 360 regions (9%) (Figure 2). Long-term cannabis users did not differ from nonusers in regional SA (Figure S2), subcortical GMVs (Table S4), or tractwise FA (Table S5). Long-term cannabis users did not differ from long-term alcohol or tobacco users on any regional brain structure measure.

Tests of dose-response associations revealed that study members with more persistent cannabis dependence had thinner cortex than those with less persistent dependence in 52 of 360 regions (14%). Study members who regularly used cannabis more persistently had thinner cortex than those who used less persistently in 41 regions (11%; Dice similarity to regions associated with dependence: 0.67). However, no associations survived adjustment for persistent dependence on other substances (Figure 3A). Persistence of cannabis dependence and persistence of regular cannabis use were not associated with regional cortical SA. Persistence of regular cannabis use, but not persistence of cannabis dependence, was associated with smaller GMVs in 4 of 10 subcortical regions: amygdala, hippocampus, thalamus, and ventral diencephalon. However, no associations survived adjustment for other substances (Figure S3). Finally, there were no

Table 2. A Comparison of Long-term Cannabis Users and Three Comparison Groups on Global Brain Measures

Brain Measure	Long-term Cannabis Users, n = 80		Comparison Group 1: Lifelong Cannabis Nonusers, n = 187		Comparison Group 2: Long-term Tobacco Users, n = 68		Comparison Group 3: Long-term Alcohol Users, n = 56		Statistical Tests of Difference Between Long-term Cannabis Users and Comparison Groups		
									LT vs. 1	LT vs. 2	LT vs. 3
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	p_{FDR} (p_{unc})	p_{FDR} (p_{unc})	p_{FDR} (p_{unc})
CT	−0.180	−0.378 to 0.019	0.216[a]	0.074 to 0.357	−0.335	−0.585 to −0.084	−0.197	−0.466 to 0.073	.007 (.002)[a]	.621 (.338)	.919 (.919)
SA	−0.135	−0.324 to 0.053	0.027	−0.095 to 0.150	−0.268	−0.459 to −0.077	0.013	−0.179 to 0.205	.209 (.155)	.621 (.337)	.497 (.295)
TBV	−0.145	−0.321 to 0.032	0.073	−0.056 to 0.202	−0.221	−0.405 to −0.037	−0.009	−0.191 to 0.174	.126 (.063)	.621 (.559)	.497 (.309)
FA	−0.097	−0.334 to 0.139	0.079	−0.052 to 0.209	−0.010	−0.222 to 0.202	−0.148	−0.418 to 0.122	.209 (.174)	.621 (.596)	.919 (.785)
WMH	0.108	−0.128 to 0.344	0.053	−0.080 to 0.187	0.194	−0.046 to 0.433	−0.077	−0.365 to 0.212	.675 (.675)	.621 (.621)	.497 (.331)
BrainAGE	0.302	0.088 to 0.516	−0.207[a]	−0.337 to −0.077	0.449	0.197 to 0.700	0.116	−0.141 to 0.373	<.001 (<.001)[a]	.621 (.383)	.497 (.277)

Means and statistical tests of group comparisons are adjusted for sex and standardized on the full sample (mean = 0, SD = 1). Raw (nonstandardized) means are given in Table S1. Group Ns are slightly lower than reported in the methods section and vary slightly owing to missing data and varying quality control exclusions for brain measures. BrainAGE is the difference between age estimated from magnetic resonance imaging data and actual chronological age.

CT, cortical thickness; FA, fractional anisotropy; LT, long-term cannabis users; p_{FDR}, false discovery rate–corrected p; p_{unc}, uncorrected p value; SA, surface area; TBV, total brain volume; WMH, white matter hyperintensity volume.

[a]FDR-adjusted (across 6 measures) statistically significant difference compared with long-term cannabis users.

associations between either continuous cannabis use measure and tractwise FA (Figure S4).

Regional Brain Structure in Long-term Alcohol and Tobacco Users

Study members with more persistent alcohol dependence had thinner cortex than those with less persistent dependence in 120 regions (33%), but only 2 survived adjustment for persistent dependence on other substances (Figure 3B). Study members with more persistent alcohol dependence did not differ significantly from those with less persistent dependence in regional cortical SA or subcortical GMVs (Figure S5); however, they had lower FA in 10 of 27 tracts (37%), including 6 that survived adjustment for other substances (Figure S6).

Study members with more persistent tobacco dependence had thinner cortex than those with less persistent dependence in 209 regions (58%), including 26 that survived adjustment for other substances (Figure 3b), along with smaller SA in 56 regions (16%), including 22 that survived adjustment for other substances (Figure S7). Study members with more persistent tobacco dependence had smaller GMV of the ventral diencephalon than those with less persistent dependence, but this association did not survive adjustment for other substances (Figure S5). These study members additionally had lower FA in 8 tracts (30%), including one that survived adjustment for other substances (Figure S6).

Secondary Analyses

We found no significant differences between long-term cannabis users and midlife recreational cannabis users or cannabis quitters (Tables S6–S9 and Figure S8).

Secondary dose-response analyses revealed that associations between persistence of tobacco dependence and global CT and brainAGE survived additional adjustment for childhood risk factors (Table S10), as did associations between persistence of alcohol dependence and regional CT in 2 regions and persistence of alcohol dependence and regional FA in 6 tracts (Figures S9 and S10). Dose-response analyses with global brain measures separated by sex revealed patterns of associations similar to those in the full sample (Table S11).

DISCUSSION

With increasing legalization and use of cannabis, it is important to examine the integrity of brain structures supporting cognitive functions in long-term users, particularly in midlife when cognitive reserves begin to shape aging trajectories. However, no clear picture of the structural brain integrity of long-term cannabis users, especially in midlife, has yet emerged, and questions remain regarding the role of polysubstance use in the manifestation of brain alterations. Here, we leveraged a large, population-representative birth cohort with comprehensive prospective substance use measures across 5 decades into midlife to address these issues. Our results advance knowledge in 3 ways.

First, long-term cannabis users had older brain age (i.e., higher brain age gap estimate) and thinner global and regional cortex than lifelong nonusers. Moreover, tests of dose-response associations revealed that people who used cannabis more persistently had older brain age and thinner
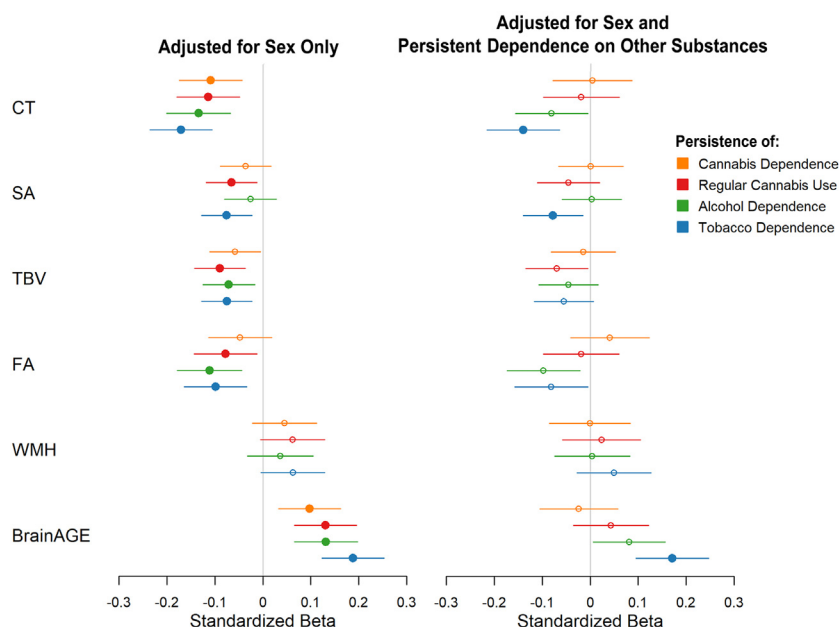
Figure 1. Visual representation of dose-response associations between persistence of cannabis, alcohol, and tobacco use from age 18 to 45 years and global measures of midlife brain structure. Standardized betas are shown with adjustment for sex only and with adjustment for sex and persistent dependence on other substances (for each of the 3 substances, this included persistence of dependence on the other 2 substances and persistent dependence on other illicit drugs). Filled circles indicate $p < .05$ after false discovery rate correction across 6 measures. BrainAGE is the difference between age estimated from magnetic resonance imaging data and actual chronological age. CT, cortical thickness; FA, fractional anisotropy; SA, surface area; TBV, total brain volume; WMH, white matter hyperintensity volume.

global and regional cortex than people who used cannabis less persistently or not at all, and further showed cannabis-related subcortical differences in areas demonstrating a high density of cannabinoid receptors, including the amygdala and hippocampus (10). However, no dose-response association was robust to adjustment for other substance use, and long-term cannabis users did not differ from long-term tobacco or alcohol users in any group comparison. These findings are consistent with recent large-sample studies (13,16) along with others failing to find cannabis-specific associations after careful control for alcohol use (18,39), though fewer cannabis studies have rigorously controlled for tobacco use. These findings are inconsistent with a recent study of 799 adolescents reporting associations between cannabis use and

thinner prefrontal cortex even after accounting for alcohol and tobacco use (40). Another study of 89 cannabis-dependent individuals from the Human Connectome Project found gray and white matter differences in comparison with nondependent individuals matched for alcohol use; however, this study was unable to also match for tobacco use (41). Our convergent findings across 2 analytic approaches for isolating cannabis effects suggest that inconsistencies reported across previous small, cross-sectional, and/or heterogeneous samples reflect, at least in part, effects of other substance use.

Importantly, our dose-response analyses were powered to detect small effects (Pearson's r of 0.095), and group comparisons were powered to detect small to medium sized differences (Supplemental Methods). Thus, it is unlikely that our
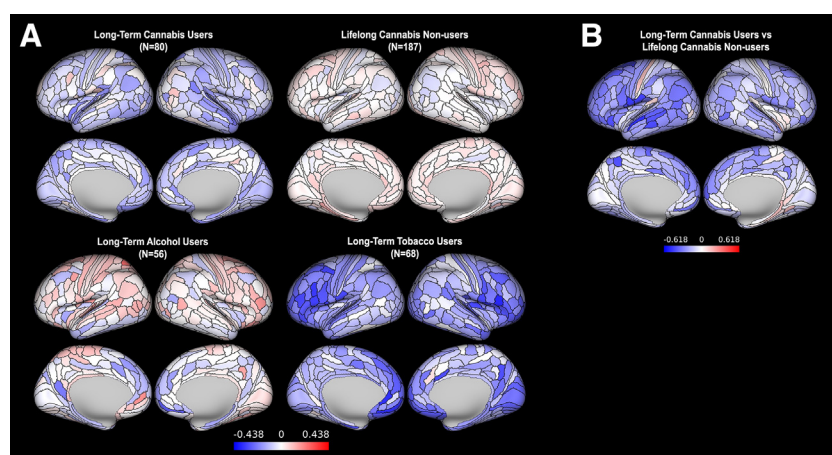

Figure 2. A comparison of long-term cannabis users and 3 comparison groups on regional cortical thickness. **(A)** Means for each group, adjusted for sex and standardized on the full sample (mean = 0, SD = 1) (blue regions are thinner than average and red regions are thicker than average). **(B)** Differences between long-term cannabis users and lifelong cannabis nonusers (34 regions were significant at false discovery rate–corrected $p < .05$, corrected across 360 regions). No other group comparisons revealed significant differences in regional cortical thickness. To allow for a comprehensive examination of effect sizes, maps have not been thresholded by significance.
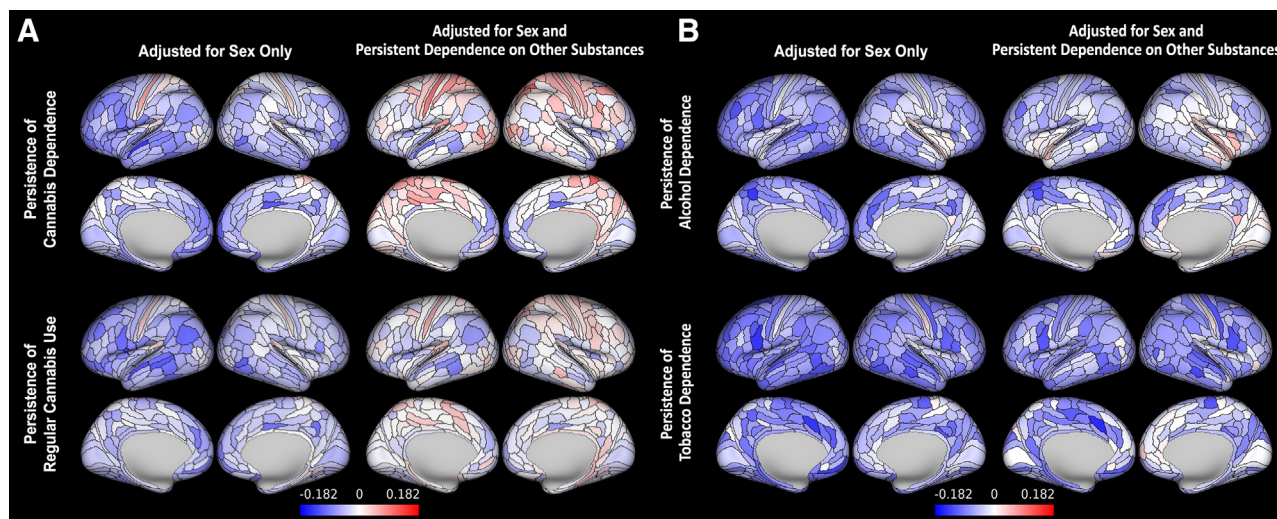
Figure 3. Dose-response associations between persistence of **(A)** cannabis use and **(B)** alcohol and tobacco dependence from age 18 to 45 years and regional measures of midlife cortical thickness before and after adjustment for persistent dependence on other substances. Colors represent standardized betas, with blues reflecting negative associations with persistence of substance use. No regions had significant associations with persistence of cannabis use after adjustment for persistent use of other substances. To allow for a comprehensive examination of effect sizes, maps have not been thresholded by significance.

analyses failed to identify even small alterations in long-term cannabis users. Furthermore, we have detected cognitive deficits in long-term cannabis users in this cohort using the strategies used here (5,6), suggesting that associations with structural brain integrity are much smaller or nonexistent. For example, we recently reported on childhood to midlife IQ declines in Dunedin Study members that were unique to long-term cannabis users and, unlike the current structural brain associations, robust to adjustment for other substance use (6). In addition, in this earlier study we specifically examined the hippocampus as an a priori region of interest, finding reduced GMVs in long-term cannabis users. Notably, this association did not survive multiple comparison correction in the present comprehensive set of analyses. Taken together, the findings from our current and earlier studies suggest not only that evidence pointing to unique effects of cannabis on the hippocampus could be explained by the limited scope of prior analyses, but also that future work including well-powered neuroimaging studies of brain function and connectivity is needed to identify links between long-term cannabis use, cognitive impairment, and brain.

Second, we found dose-response associations between persistence of alcohol and tobacco dependence and structural brain integrity that were larger and more robust to covariate adjustment than those for cannabis use. Dose-response associations between persistence of alcohol dependence and both older brain age and thinner cortex had larger effect sizes than those for cannabis use. Persistence of alcohol dependence was additionally marginally associated with global white matter microstructural integrity and was significantly associated with regional integrity in 6 white matter tracts even after covariate adjustment. These findings are consistent with studies reporting gray matter differences in alcohol users but not in cannabis users (16,39,42) as well as studies consistently

reporting associations between alcohol use and widespread alterations in white matter (43–45). For tobacco use, we found that continuous measures of use were more strongly associated with older brain age and thinner cortex than either cannabis or alcohol use, surviving adjustments both for other substance use and for childhood risks at the global level. Although there is evidence for associations between tobacco use and gray matter (46–49), our study represents one of few studies examining cannabis, alcohol, and tobacco use in the same cohort, and the dominant effect of tobacco use (rather than cannabis or alcohol) is relatively novel (16,50). This pattern is also consistent with a recent UK Biobank study reporting a slightly larger association between older brain age and tobacco use than that with alcohol use (51) (cannabis use was not assessed).

Finally, because most studies of cannabis use and the brain have been conducted in adolescents and young adults with varying levels of use, our study shines much-needed light on the associations between substantial long-term cannabis use and brain structure in midlife, which has emerged as a critical platform in shaping how individuals experience aging in later life (52). It is reasonable to expect that effects of long-term cannabis use on the brain would be easier to detect in an older sample of persistent users, given accumulated use over many years. Indeed, we observed clear patterns of associations between long-term cannabis use and both thinner cortex and older brain age, but these were entirely explained by other substance use, suggesting that there may not be mechanisms specific to the endocannabinoid system whereby cannabis use is associated with lasting alterations in structural brain integrity. Our comparatively robust finding of older midlife brain age in persistent tobacco users and, to a lesser extent, persistent alcohol users is particularly relevant for ongoing efforts to ameliorate the impact of age-related diseases, given that

individuals with older brain age have increased risk for negative outcomes including accelerated cognitive decline and ADRDs (29,31,32).

Our study has limitations. First, we lack structural brain integrity measures preceding initiation of cannabis use because this cohort's childhood predates neuroimaging technology. Future longitudinal neuroimaging studies will be essential for characterizing possible causal relationships between cannabis use and brain structure and for ruling out preexisting alterations, because current evidence for structural brain alterations in cannabis users preceding onset of use is mixed (40,53–55). Second, cannabis use was self-reported as past-year number of days used, and a more fine-grained measure of exposure could have increased sensitivity and better captured patterns of polysubstance use. In addition, biological assays could have helped detect under-reporting, though under-reporting for fear of admitting to illegal drug use is unlikely because study members, interviewed repeatedly over a lifetime, have learned to trust the study's confidentiality guarantee (56). Third, high rates of polysubstance use prevented testing for effects specific to users of cannabis alone. However, dose-response analyses allowed for isolation of cannabis effects in more typical cannabis users through covariate control. Fourth, because all associations between cannabis use and brain structure were explained by other substance use, we do not provide an in-depth analysis on further potential confounds. Future work should seek to better understand relationships between cannabis use and other factors such as antecedents to substance use, comorbid illness, and genetic predisposition (19). Fifth, though secondary analyses revealed no differences between long-term cannabis users and cannabis quitters, an in-depth analysis of abstinence was additionally outside the scope of this work, and future work is needed to better understand the effects of cessation. Finally, our findings are based on data collected from a single New Zealand birth cohort who began using cannabis in the 1970s and 1980s. While Dunedin Study findings generally match findings from U.S. samples, concentrations of Δ^9-tetrahydrocannabinol, the primary psychoactive constituent of cannabis, have risen in recent years (57). Therefore, if Δ^9-tetrahydrocannabinol underlies associations, then cannabis-related brain differences reported here might reflect underestimates.

In summary, the detailed prospective substance use measures in our large dataset allowed for more accurate quantification of drug use than many existing studies, providing a uniquely comprehensive picture of the relative strengths of associations between cannabis, tobacco, and alcohol use with midlife structural brain integrity. This picture revealed that long-term cannabis users by midlife have widely distributed alterations in brain structure including thinner cortex, lower subcortical GMVs, and older brain age. Critically, however, all midlife structural brain alterations in long-term cannabis users were explained by their propensity to also use tobacco and alcohol, which had relatively outsized effects on brain structure. Thus, our findings collectively suggest that long-term cannabis use is not likely independently associated with midlife structural brain integrity. This emphasizes the importance of carefully accounting for polysubstance use in future studies of cannabis, brain, and behavior along with identifying convergent cellular and molecular pathways through which cannabis, alcohol, and tobacco exert effects on the brain. These findings may further help inform policy makers and health care providers as they weigh the effects of long-term cannabis use relative to other substances and seek to identify optimal policies for legalization and strategies for intervention, including mitigating risk for later-life ADRDs.

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