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Testing associations between cannabis use and subcortical volumes in two large population-based samples

Nathan A. Gillespie, PhD[1,2], **Michael C. Neale, PhD**[1], **Timothy C. Bates, PhD**[3], **Lisa T. Eyler, PhD**[4,5], **Christine Fennema-Notestine, PhD**[5], **Jasmin Vassileva, PhD**[6], **Michael J. Lyons, PhD**[7], **Elizabeth C. Prom-Wormley, MPH PhD**[8], **Katie L. McMahon, PhD**[9], **Paul M. Thompson, PhD**[9], **Greig de Zubicaray, PhD**[10,11], **Ian B. Hickie, MD**[12], **John J. McGrath, MD, PhD**[13], **Lachlan T. Strike**[2,9,10], **Miguel E. Rentería**[2], **Matthew S. Panizzon, PhD**[5], **Nicholas G. Martin, PhD**[2], **Carol E. Franz, PhD**[5], **William S. Kremen, PhD**[5,*], and **Margaret J. Wright, PhD**[2,9,*]

[1]Virginia Institute for Psychiatric and Behavior Genetics, Virginia Commonwealth University, VA, USA

[2]QIMR Berghofer Medical Research Institute, QLD, Australia

[3]Department of Psychology, University of Edinburgh, EH8 9JZ, UK

[4]Desert-Pacific Mental Illness Research, Education, and Clinical Center, VA San Diego Healthcare System, CA, USA

[5]Department of Psychiatry, University of California San Diego, CA, USA

[6]Institute for Drug and Alcohol Studies, Virginia Commonwealth University, VA, USA

[7]Department of Psychological and Brain Sciences, Boston University, Boston, MA, USA

[8]Department of Family Medicine and Population Health, Virginia Commonwealth University, VA, USA

[9]Centre for Advanced Imaging, The University of Queensland, QLD, Australia

[10]School of Psychology, The University of Queensland, QLD, Australia

[11]Faculty of Health and Institute of Biomedical Innovation, Queensland University of Technology

[12]Brain and Mind Research Institute, University of Sydney, NSW, Australia

[13]Queensland Brain Institute, The University of Queensland, QLD, Australia

[14]VA San Diego Center of Excellence for Stress and Mental Health, CA, USA

Abstract

Correspondence: Nathan A. Gillespie, Virginia Commonwealth University, Virginia Institute for Psychiatric and Behavioral Genetics, Box 980126, Richmond, VA 23298-0126 (USA) ngillespie@vcu.edu.

*Dr Kremen and Dr Wright are joint senior authors

Competing interests

We declare no competing interests defined as those of a financial nature that, through their potential influence on behavior or content, or from perception of such potential influences, which could undermine the objectivity, integrity or perceived value of this publication.

BACKGROUND—Cannabis use is potentially hazardous for developing brains. Disentangling the putative impact of cannabis on brain morphology from other comorbid substance use is critical. After controlling for the effects of nicotine, alcohol and multi-substance use, we hypothesized that frequent cannabis use is associated with significantly smaller subcortical grey matter volumes.

DESIGN—Exploratory analyses using mixed linear models, one per region of interest (ROI), were performed whereby individual differences in volume (outcome) at seven subcortical ROIs were regressed onto cannabis and comorbid substance use (predictors).

SETTING—Two large population-based twin samples from the United States and Australia.

PARTICIPANTS—622 young Australian adults (66% female; μ_{age} = 25.9, SD=3.6), and 474 middle-age U.S. males ($\mu_{age} = 56.1_{SD=2.6}$) of predominately Anglo-Saxon ancestry with complete substance use and imaging data. Subjects with a history of stroke or traumatic brain injury were excluded.

MEASURES—Magnetic resonance imaging (MRI) and volumetric segmentation methods were used to estimate volume in seven subcortical ROIs: thalamus; caudate nucleus; putamen; pallidum; hippocampus; amygdala; and nucleus accumbens. Substance use included maximum nicotine and alcohol use, total lifetime multi-substance use, maximum cannabis use in the young adults, and regular cannabis use in the middle-age males.

FINDINGS—After correcting for multiple testing (p=0.007), cannabis use was unrelated to any subcortical ROI. However, maximum nicotine use was associated with significantly smaller thalamus volumes in middle-age males.

CONCLUSIONS—This is the largest study integrating brain imaging, self-report cannabis and comorbid substance use data. In these exploratory analyses based on young adult and middle age samples, normal variation in cannabis use is statistically unrelated to individual differences in brain morphology as measured by subcortical volume.

Keywords

cannabis use; multi-substance use; imaging; gray matter; subcortical; brain volume

Introduction

Cannabis is commonly used by adolescents and young adults(1), and if used frequently can be potentially hazardous to mental health. During development, there are dynamic changes in brain neurochemistry, fibre architecture, and tissue composition (2), which could be impacted by chronic cannabis use (CU), or comorbid substance use (SU) such as nicotine and alcohol (3). In view of changing cultural norms, and expanding cannabis medicalization and decriminalization, disentangling the potential impact of cannabis on brain morphology from other substances is critical.

In contrast to the psychiatric and social consequences of cannabis use (4), our knowledge of the morphological changes associated with cannabis use is not well characterized. Whereas infrequent or regular adult cannabis use does not appear to affect neurological functioning

(5), chronic cannabis use appears to affect cognition in adults (5). Among adolescents and young adults, cannabis use is associated with enduring cognitive decline(6). This suggests that cannabis use likely affects or is associated with changes in brain morphology.

Grey matter volume (GMV) is a widely-used indicator of brain morphology. We reviewed 24 studies (7–30), one review (31), and one meta-analysis (32) examining GMV and SU. Varying by region, six studies reported greater GMV related to SU (10, 13, 15, 28, 29), one found no difference (14), while the remainder identified smaller GMVs in relation to more frequent SU. Among the regions of interest (ROI), the putamen, hippocampus, and thalamus subcortical structures have emerged as putative markers for SU. However, findings have been equivocal, and vary by substance. We reviewed eight reports identifying smaller putamen volumes among heavy alcohol (8, 9, 22), nicotine (12), cannabis (24), cocaine (17, 23), and ecstasy (33) users, compared to three reports identifying larger putamen volumes among methamphetamine (10) and nicotine users (28, 29). Ten reports have identified reductions in hippocampus volume associated with alcohol (9, 25), nicotine (12, 34), methamphetamines (26), and cannabis (7, 27, 30, 34, 35), compared to one report that found no association with alcohol use (14). Several reports have also linked smaller thalamus volume to increased alcohol (8, 22), nicotine (28), methamphetamines (26), and opioid (18) use compared to one that identified larger thalamus volumes among cannabis users (13).

The above studies vary widely in terms of their image acquisition, selection of regions, volume estimation methods, and statistical control for comorbid SU. Because cannabis use is highly comorbid with a variety of licit and illicit substance use (36), the need to disentangle the putative effects of alcohol, nicotine or multi-substance use (MSU) is critical. For example, alcohol use is associated with smaller hippocampus, thalamus, putamen and palladium volumes (8, 9, 22, 25), whereas studies investigating the associations between nicotine use and subcortical volumes are equivocal (12, 28, 29, 37). Less is known about the effects of poly or multi-substance use (MSU) (38), with evidence suggestive smaller subcortical volumes in regions such as the thalamus (39), and the putamen (40). However, another major limitation is sample size. In 21 reports investigating associations between licit or illicit SU and subcortical volumes in one or more regions, the average sample size was 90 (8–10, 12–15, 17, 18, 22–29, 35, 41–43). Consequently, larger imaging samples that include measures of cannabis and comorbid SU are required.

The aim of this report is to determine the size of associations between cannabis use and the volumes of seven subcortical ROIs in two independent population-based samples. We hypothesize that increased cannabis use will be associated with smaller subcortical volumes over and above the effects of comorbid nicotine, alcohol and lifetime multi-substance use.

Methods

Design

Our approach relied on data from two samples with similar phenotypic measures. We began by measuring the strength of associations between nicotine, alcohol, multi-substance use, cannabis use and subcortical volumes at seven regions of interest (ROIs). We performed exploratory analyses to determine the relationship between cannabis use and volume.

Specifically, we regressed each subcortical ROI onto nicotine, alcohol, multi-substance, and cannabis use using mixed linear models. For each sample, we fitted one regression for every ROI. All results were adjusted for multiple testing.

Sample 1

Participants—Sample 1 comprised 622 young male and female adult twins from the ongoing population-based Brisbane Longitudinal Twin Study (BLTS) (44, 45). The participants were of European ancestry, predominately Anglo-Saxon, who were ascertained beginning 1992 to study of melanocytic naevi, and have since been followed up on multiple occasions.

Procedure—Between 2009–2015 the BLTS subjects participated in an online survey of substance use (66% female; μ_{age} = 25.9, SD=3.6, range=18–38) (44, 45). Almost three years prior, the participants were scanned with magnetic resonance imaging (MRI) (μ_{age} = $23.0_{SD=2.8}$, range=18–30) as part of the Queensland Twin Imaging study (46). There were N=27 and N=29 subjects whose onset ages at cannabis initiation and heaviest cannabis use respectively occurred after scanning. These subjects were excluded. Only subjects whose age at cannabis initiation or age at heaviest cannabis use preceded or occurred during the scanning year were included in the analyses. Informed consent was obtained from all participants who received an honorarium of AUD$50 for completion of the survey, and $100 for MRI participation to defray travel costs.

Measures

Substance use: The online survey included ***maximum cannabis use***, which assessed the time or times when cannabis was used the most (never used, once or twice, monthly, weekly, and daily or almost daily), ***maximum nicotine use*** based on the total number of cigarettes smoked lifetime (never used, 1–2 times, 3–5 times, 6–10 times, 11–15 times, 16–19 times, 20–25 times, 26–99 times, 100–199 times, and ≥ 200 times), ***maximum alcohol use*** based on the period when drinking the most how often subjects consumed 4 (female) or 5 (male) or more drinks at least once a week for a month or more, and total ***lifetime multi-substance use* (MSU)** based on having ever tried or used the following 9 substances: cocaine; amphetamine-type stimulants (speed, ice, diet pills, etc.); inhalants (nitrous, glue, petrol, paint thinner, etc.); sedatives or sleeping pills (valium, serepax, Rohypnol, etc.); hallucinogens (LSD, acid, mushrooms, PCP, etc.); opioids (heroin, morphine, methadone, codeine, etc.); ecstasy, ketamine, GHB or party drugs (E, X, MDMA, K, Special K, Fantasy); over the counter/prescription painkillers and analgesics for non-medical purposes (e.g. cough medicine, mersyndol, ibuprofen, panadol, panadeine, codeine, hydrocodone etc); and over the counter/prescription stimulants for non-medical purposes (e.g. no doze, pseudoephedrine, dexamphetamine, Ritalin etc). In the Supplement, we demonstrate that the construct of lifetime multi-substance use is psychometrically homogenous, possesses good internal reliability, and concurrent validity. We also show that familial aggregation in multi-substance use is entirely attributable genetic risk factors shared between siblings and account for 51% of the total variance (Table S1). All other substance use descriptives are shown Table 1.

Imaging: Described in detail elsewhere (47) MRI images were acquired on a 4T Bruker Medspec Scanner at the Center for Magnetic Resonance, University of Queensland, Australia using an inversion recovery rapid gradient echo protocol. Total intracranial volume and the volumes of 14 subcortical structures were extracted: thalamus; Caudate Nucleus; putamen; pallidum; hippocampus: amygdala; and nucleus accumbens. Quality of delineation was assessed following the Enhancing Neuro-Imaging Genetics through Meta-Analysis consortium protocol for subcortical structures (http://enigma.loni.ucla.edu/protocols/imaging-protocols/quality-checking-subcortical-structures), which resulted in the exclusion of 1.83% of volumes segmented with Freesurfer (Version 5.3). As discussed by Fischl (48), images were skull stripped, transformed to Talairach space and a probabilistic atlas is used to assign each voxel a neuroanatomical label. Prior to scanning, all participants were screened by self-report for imaging suitability, including significant medical, psychiatric, or neurological conditions (including head injuries), and current use of psychoactive medication. As shown in Table 2, in Sample 1 the correlations between the mean volumes of the homologous left and right subcortical ROIs were high and ranged from 0.60 to 0.93. Therefore, we averaged the left and right homologous ROIs and analyzed the residuals after adjusting for age, and total intracranial volume. Because of the higher prevalence of cannabis use among males (49), residuals were also adjusted for sex.

Sample 2

Participants—Sample 2 comprised 474 middle-age male twins from the population-based Harvard Drug Study (HDS) (50) who were scanned with MRI as part of the Vietnam Era Twin Study of Aging (VETSA) between 2003–2007 (51, 52). Participants were concordant for US military service at some time between 1965–1975. Nearly 80% reported no combat experience. The sample is 88.3% non-Hispanic white, 5.3% African-American, 3.4% Hispanic, and 3.0% “other” participants. Based on data from the US National Center for Health Statistics, the sample is very similar to American men in their age range with respect to health and lifestyle characteristics (53). Written informed consent was obtained from all participants. The local ethics committee approved the study.

Procedure—Phenotypic data were collected as part of the HDS in 1992 ((μ_{age}=44.6, SD=2.5) by telephone interview from members of the Vietnam Era Twin Registry, comprising male twin pairs who served in the U.S. military between 1965 and 1975 (50). The VETSA is a longitudinal behavioural genetic study with a primary focus on cognitive and brain ageing in men. It comprises a subset of over 1,200 twins from the Vietnam Era Twin Registry (51). A companion VETSA project included the administration of MRIs to a subset of participants twice. MRI data for this report came from the first MRI (VETSA1) in which twins (μ_{age}=56.1, SD=2.6, range=51.1–60.2) underwent 3D structural MRIs to measure cortical and subcortical volumes, cortical thickness and surface area. The minimum difference between age at first cannabis initiation and scanning was 20.2 years. Exclusion criteria included stroke, traumatic brain injury (TBI) and brain tumours. A total of 14 and 36 subjects who reported stroke and TBI respectively at the time of scanning were excluded from our analyses.

Measures

Substance use: The HDS in 1992 assessed ***regular cannabis use*** based on having ever used regularly once per week or more (0=No, 1=Yes). All never users were coded as zero. Other substance use measures included ***maximum nicotine use*** based on the number of cigarettes smoked per day during heaviest period (never used, 1–2 times, 3–5 times, 6–10 times, 11–15 times, 41–99 times), ***maximum alcohol use*** based on the number of days drinking per month when drinking the heaviest, and ***lifetime multi-substance*** use based on having ever tried the following 5 substances: stimulants; sedatives; cocaine; heroin; and PCP or other psychedelics. Substance use descriptives are shown Table 1.

Imaging: Between 2003–2007, the VETSA (51) acquired brain imaging on Siemens 1.5 Tesla scanners at University of California, San Diego, and at Massachusetts General Hospital. Sagittal T1-weighted MPRAGE sequences were employed with the following acquisition parameters: TI=1000ms, TE=3.31ms, TR=2730ms, flip angle=7 degrees, 13 slice thickness=1.33mm, voxel size 1.3×1.0×1.3mm. Images were automatically corrected for spatial distortion caused by gradient nonlinearity and B1 field inhomogeneity. Two T1-weighted images per subject were registered and averaged to improve signal-to-noise. Volumetric segmentation (54, 55) methods were based on FreeSurfer (FS Version 3.0.1b). The semi-automated, fully 3D whole-brain segmentation procedure uses a probabilistic atlas and applies a Bayesian classification rule to assign a neuroanatomical label to each voxel (56). A widely-used training atlas has been shown to be comparable to that of expert manual labelling (56), but we created a VETSA-specific atlas that further increased accuracy compared to expert manual labelling (57).

As shown in Table 2, in Sample 2 the correlations between the mean volumes of the homologous left and right subcortical ROIs ranged from 0.54 to 0.90. Again, we averaged the left and right homologous ROIs, and analyzed the residuals after adjusting for age, total intracranial volume, and MRI site.

Statistical analyses

Measures of association—Measures of association between substance use and the volume at each ROI were based on polyserial correlations estimated using the OpenMx software package (58) in $R_{3.1.1}$ (59). Polyserial correlations represent the inferred latent correlations between the continuous sub-cortical volumes and the ordered categorical SU variables.

Mixed linear models—To determine the contribution of cannabis and comorbid substance use to volume we fitted mixed linear models. Specifically, all models were conducted in a multilevel framework, using the *lme* function from the *nlme* package (60). Our rationale was to model the random effects to adjust for the presence of correlated observations in twin data. In each model, family ID and zygosity to denote whether twins were part of a genetically identical monozygotic or dizygotic twin pair were entered as the random effect. For each sample, we then performed 7 regressions using a corrected p-value threshold of 0.007.

Results

Sample 1

Across sex, the average age at cannabis initiation was $17.7_{SD=2.8}$ in the young adults, while the average age at which cannabis was used the most was $18.2_{SD=4.11}$. The number of pairwise observations, along with polychoric correlations is shown in Table 3. Depending on the region, the number of subjects with complete volume and maximum cannabis use data ranged from 618 to 622. Correlations between subcortical volumes and the cannabis use measures were all small, ranging from r = −0.06 to r = +0.05, each with relatively large standard errors. The correlations between each of the subcortical regions and nicotine, alcohol and multi-substance use (MSU) were also small. Among the larger negative correlations, maximum alcohol and multi-substance use were ach associated with smaller hippocampus volume (r=−0.07). A lifetime history of greater multi-substance use was also associated with a smaller pallidum (r=−0.08) volume.

Mixed model linear regression results for the middle-age males appear in Table 4. Commensurate with the polychoric correlations, maximum cannabis use was unrelated to volume at each ROI. There were, however, nominal associations ($p<0.01$) between smaller hippocampus volumes and increased multi-substance use as well as maximum cannabis use.

Sample 2

In the middle-age males, the average age at cannabis initiation was $20.2_{SD=3.5}$, while the average age at which they first used cannabis more than five times was $20.4_{SD=3.1}$. The number of pairwise observations and polychoric correlations are shown in Table 5. Depending on the region, the number of participants with complete cannabis use data ranged between 463 and 474. All correlations between subcortical volumes and the cannabis use measures were small and ranged from −0.15 to +0.05, and all had relatively large standard errors. Among the higher negative correlations, maximum nicotine use was associated with smaller putamen (r=−0.12) and thalamus volumes (r=−0.15). Maximum alcohol use was associated with smaller hippocampus volumes (r=−0.15).

Mixed model linear regression results for the middle-age males appear in Table 4. Commensurate with the polychoric correlations, increased levels of maximum nicotine use were significantly associated with smaller thalamus volumes ($\beta = -0.15$, $p = 0.003$). Nominal associations were observed between increased levels of maximum nicotine use and smaller putamen volumes ($p<0.05$), as well as between increased maximum alcohol use and smaller hippocampus volumes ($p<0.10$).

Discussion

This is the largest exploratory analysis integrating brain imaging with self-report cannabis and comorbid substance use (SU) data. After correcting for multiple testing, there was no effect of cannabis use (CU) on the volume at any subcortical region of interest (ROI) in young adults or middle-aged males. However, increased lifetime maximum nicotine use significantly predicted smaller thalamus volumes in middle-age males. In the context of expanding medicalization and decriminalization and the concerns surrounding the

consequences of increased cannabis availability, our findings suggest that normal variation in cannabis use is statistically unrelated to brain morphology as measured by subcortical volumes in non-clinical samples.

Our results do not support a recent finding showing that lifetime cannabis use is associated with reduced amygdala volumes (61). Using a sample comparable to our young adults in terms of age and size, Pagliaccio et al. (61) regressed the volumes of subcortical ROIs onto lifetime cannabis use along with the covariates of sex, ethnicity, zygosity, household income, and intelligence, and found that amygdala volumes among cannabis users were 2.3% smaller. However, when corrected for comorbid SU the study's main effect of cannabis use on left amygdala volume declined to the threshold of p=0.02, which is not significant when adjusted for multiple testing. Among reports identifying reduced hippocampus volumes in cannabis users, sub-clinical measures of comorbid SU were either not included (27), or the covariates were limited to alcohol and nicotine (35). Similarly, Yip et al's (24) marginal association between cannabis dependence and smaller putamen volumes did not model comorbid nicotine use or sub-clinical forms of abuse-dependence. Even if we ignore comorbid substance use, the polychoric correlations illustrate that the effect sizes of cannabis use on volume at each ROI remain small and account for very little covariance.

Because our analyses were exploratory, we employed a Bonferroni corrected p-value threshold of 0.007. There was a nominal association between increased cannabis use and smaller hippocampus volumes in the young adult sample. One might predict that the lack of any statistically significant main effect of cannabis use or other substances in the young adults is indicative of insufficient cumulative exposure to the detrimental effects prior to scanning. Yet, there was no effect of cannabis use among the middle-aged males who ought to have had longer cumulative exposure. Instead, only maximum nicotine use significantly predicted smaller thalamus volumes in the middle-age males. If typical cigarette smoking is in the range of 20–40 per day, and if cannabis smoking is 10–20% of this quantity, then cannabis use is unlikely to result in any detectable volumetric differences. Of course, a longer exposure to cannabis *per se* may not predict volume if initiation occurred after a developmentally sensitive period. Battistella et al. (7) found marginally larger GMV reduction among early cannabis initiators. Striatal plasticity peaks during adolescence (62). Therefore, a combination of early cannabis initiation and frequent cannabis use could result in reduced GMV due to plasticity loss at excitatory synapses (63).

We recommend caution when directly comparing the findings between samples. In addition to the different imaging methods techniques employed, there are measurement artefacts, as well as sex and cohort differences in SU (64). For example, multi-substance use in the young adults was based on substances including methamphetamine - a resurgent drug (65), and non-medical use of prescription stimulants and analgesics – a recent phenomenon (66), versus multi-substance use in the middle-aged males whose rates of cocaine, sedative and stimulant use were likely higher (64). Maximum nicotine use was assessed differently in each sample; total lifetime use in the younger adults versus daily number of cigarettes 'when using the most' in the middle-age males. Data harmonization is required before direct comparisons can be made. Regarding sex differences, although the literature now supports sexual dimorphism(67), larger samples are again required to test for these effects. We

nevertheless re-ran all seven mixed linear models, and each case, neither the main effect of sex nor the interaction between sex and maximum cannabis was significant at our corrected p-value of 0.007. There was however, a nominal interaction between sex and cannabis (β = −0.13, p = 0.07) for the putamen.

Regarding the effect of MSU, it was only nominally predictive of smaller pallidum and hippocampus volumes in the young adults. This is inconsistent with Rodrigues et al. (68) who found that the cannabinoid subtype-1 and the μ-opioid receptors are targeted to some of the same postsynaptic neurons in the rat putamen-Caudate nucleus. The putamen and Caudate form the dorsal striatum, which in addition to coordinating body movements, is involved in reward and decision-making, notably in relation to sensitivity to reward and habit formation (69). In a covariance analysis of subcortical volumes, we have previously identified four distinct genetic factors, including a basal ganglia/thalamic factor comprising the putamen, Caudate, pallidum, and thalamus (70). To the extent that striatal morphology may serve as a biomarker for neurodegenerative disease via substance use in general, this was not supported by our results.

Although nicotine did not significantly predict putamen volume in middle-age males (p=0.04), the nicotine-putamen correlation was nevertheless among the highest (r=0.12). When considered with the significant nicotine-thalamus association, these results are consistent with prior findings. For example, Froeliger et al.(12) found that smoking abstinence was associated with higher pre-quit GMV in the putamen as well as the hippocampus. Vafaee et al. (71) observed significant global impairment in terms of cerebral blood flow and metabolic rate of oxygen in abstaining smokers in the left putamen and thalamus. Other studies have reported associations between nicotine phenotypes and the thalamus (28, 72). The putamen, thalamus, and hippocampus all contain large numbers of neuronal nicotinic acetylcholine receptors (73), which have been associated with risk for nicotine dependence during adulthood (74).

Limitations

Our findings must be interpreted in the context of four potential limitations. First, compared to the middle-age males, the young adult sample was scanned much closer to the mean ages of cannabis initiation and period of heaviest use. Because assessment age can bias recollection (75), more accurate recall is expected in the younger participants. It is plausible that the absence of any significant findings in these young adults can be attributed to their not having accumulated sufficient exposure to the putative detrimental effects of substance use on brain morphology. Second, this study examined subcortical regions of interest. Hence, our results should not be generalized to other brain morphologies, including individual differences in cortical regions. Third, multi-substance use was based on the total number of substances ever tried lifetime, not including nicotine, alcohol and cannabis. The association between our validated measure of multi-substance use (see Supplement) and volume may be driven by the frequency and quantity of use of one or more of these substances. In follow-up hierarchical regression analyses, measures of frequency of use for each of these covariates were not associated with volume at any ROI. Finally, our data were neither experimental nor longitudinal. It is possible that smaller subcortical volumes

predispose individuals to increased SU. In the case of the middle-age males, it is plausible that having a smaller thalamus is a causal risk factor for greater nicotine use. Commensurate with this idea, Squeglia et al. (76) found that pre-existing volume differences in frontal brain regions predicted future alcohol use, including further volume reductions in alcohol using teenagers. Pre-existing morphological differences could also arise in utero because of maternal SU (77). Given the observed associations between brain volume and executive functioning (78), future modelling that includes tests of causal hypotheses, versus non-causal but correlated genetic risks should be a public health priority. Such data would enable individuals to make informed cost-benefit judgements regarding the consequences of SU, as well influence rational law making.

Conclusion

In the context of expanding medicalization and decriminalization and concerns surrounding the consequences of increased availability, cannabis use is unrelated to any subcortical region of interest. However, maximum nicotine use was associated with significantly smaller thalamus volumes, but only in middle-age males. Other MRI phenotypes such as cortical and white matter measures need to be investigated and the putative associations between cortical regions and substance use explored. MRI measures combined with genetically informative cross-panel longitudinal designs (79) are necessary to resolve critical questions of causality, sources of genetic and environmental covariance, and whether or not the putative causal effects of SU on brain morphology are reversible. The recently announced NIH program “Adolescent Brain and Cognitive Development”, which plans to prospective study 10,000 youth aged 10–20 years has the potential to explore the hypotheses generated by our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Distribution of substance use measures for the young adults (Sample 1) and the middle-age males (Sample 2).

	Sample 1		Sample 2
	Males	***Females***	***Males***
Age of cannabis initiation	**u=17.5yrs**	**u=17.8yrs**	**u=20.2yrs**
	SD=2.8	**SD=2.8**	**SD=3.5**
	range=10–32	**range=12–32**	**range=13–38**
Maximum cannabis use			
When using the most how often did you use it?			
Never used	370	678	-
Once or twice	258	324	-
Monthly	52	45	-
Weekly	64	44	-
Daily or almost daily	94	56	-
Regular Cannabis use			
Have you ever used marijuana regularly once per week or more?			
No	-	-	359
Yes	-	-	115
Maximum alcohol use			
When drinking the most how often did you consume ≥4 (female) or ≥5 (male) drinks at least once a week for a month or more?			
Never drank	11	23	-
Consumed <4 (female) / <5 (male) drinks	312	528	-
Consumed ≥4 (female) / ≥5 (male) drinks	676	797	
Number of days drinking per month when drinking the heaviest:	-	-	u=10.0 days
			SD=9.3
			range=0–30
Maximum nicotine use			
Total number of cigarettes smoked lifetime:			
Never	374	678	-
1–2 times	66	78	-
3–5 times	61	79	-
6–10 times	39	55	-
11–15 times	28	33	-
16–19 times	12	19	-
20–25 times	35	30	-
26–99 times	64	68	-
100–199 times	39	47	-
≥200 times	281	263	-
Cigarettes per day when smoking the most:			
Never	-	-	189

	Sample 1		Sample 2
	Males	***Females***	***Males***
Age of cannabis initiation	**u=17.5yrs**	**u=17.8yrs**	**u=20.2yrs**
	SD=2.8	**SD=2.8**	**SD=3.5**
	range=10–32	**range=12–32**	**range=13–38**
1–15	-	-	56
16–20	-	-	74
21–30	-	-	67
31–40	-	-	57
≥41	-	-	32
Multi-substance use	u=1.4	u=1.1	u=0.7
	SD=2.1	SD=1.8	SD=1.4
	range=0–10	range=0–10	range=0–5

Table 2.

Polyserial correlations (and their standard errors) between homologous left and right subcortical regions of interest.

	Sample 1	Sample 2
1. putamen volume	$0.89_{(0.01)}$	$0.85_{(0.02)}$
2. caudate volume	$0.93_{(0.00)}$	$0.90_{(0.01)}$
3. pallidum volume	$0.64_{(0.02)}$	$0.66_{(0.03)}$
4. hippocampus volume	$0.86_{(0.01)}$	$0.71_{(0.03)}$
5. amygdala volume	$0.65_{(0.02)}$	$0.60_{(0.04)}$
6. accumbens volume	$0.60_{(0.02)}$	$0.54_{(0.04)}$
7. thalamus volume	$0.88_{(0.01)}$	$0.73_{(0.03)}$

Table 3.

The number of pairwise observations (upper diagonal), polychoric correlations and (standard errors) in the young Australian adults (Sample 1). Correlations between substance and volumes are shaded.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. Maximum cannabis use	1985	1977	1984	1985	622	618	622	622	621	622	622
2. Maximum nicotine use	0.61$_{(0.01)}$	2346	2346	2346	762	758	762	762	760	762	762
3. Maximum alcohol use	0.29$_{(0.02)}$	0.32$_{(0.02)}$	2366	2366	770	766	770	770	768	770	770
4. Multi-substance use	0.62$_{(0.01)}$	0.44$_{(0.02)}$	0.25$_{(0.02)}$	2862	849	845	849	849	847	849	848
5. putamen volume	0.03$_{(0.04)}$	0.00$_{(0.04)}$	0.00$_{(0.04)}$	−0.07$_{(0.04)}$	849	845	849	849	847	849	848
6. caudate volume	0.02$_{(0.05)}$	0.00$_{(0.04)}$	0.02$_{(0.04)}$	0.00$_{(0.04)}$	0.35$_{(0.03)}$	845	845	845	843	845	844
7. pallidum volume	−0.04$_{(0.04)}$	−0.04$_{(0.04)}$	−0.04$_{(0.04)}$	−0.08$_{(0.03)}$	0.11$_{(0.03)}$	0.35$_{(0.03)}$	849	849	847	849	848
8. hippocampus volume	−0.01$_{(0.04)}$	−0.04$_{(0.04)}$	−0.07$_{(0.04)}$	−0.07$_{(0.04)}$	0.17$_{(0.03)}$	0.11$_{(0.03)}$	0.26$_{(0.03)}$	849	847	849	848
9. amygdala volume	0.04$_{(0.04)}$	0.02$_{(0.04)}$	0.00$_{(0.04)}$	0.00$_{(0.04)}$	0.29$_{(0.03)}$	0.17$_{(0.03)}$	0.22$_{(0.03)}$	0.37$_{(0.03)}$	847	847	846
10. accumbens volume	−0.01$_{(0.04)}$	−0.01$_{(0.04)}$	0.00$_{(0.04)}$	0.01$_{(0.04)}$	0.17$_{(0.03)}$	0.29$_{(0.03)}$	0.25$_{(0.03)}$	0.15$_{(0.03)}$	0.29$_{(0.03)}$	849	848
11. thalamus volume	0.02$_{(0.04)}$	0.05$_{(0.04)}$	−0.01$_{(0.04)}$	0.01$_{(0.04)}$	0.35$_{(0.03)}$	0.17$_{(0.03)}$	0.31$_{(0.03)}$	0.35$_{(0.03)}$	0.21$_{(0.03)}$	0.15$_{(0.03)}$	848

Note: Maximum cannabis use = frequency of cannabis use when using the most (never used, once or twice, monthly, weekly, and daily or almost daily); Maximum nicotine use = total lifetime cigarettes ever smoked; Maximum alcohol use = when drinking the most have consumed 4 (female) or 5 (male) or more drinks at least once a week for a month or more; Multi-substance use = total lifetime use of cocaine, amphetamines, inhalants, sedatives, hallucinogens, opioids, ecstasy (including ketamine, GHB or party drugs), non-medical use of over the counter/prescription analgesics, and non-medical use of over the counter/prescription stimulants.

Table 4.

Standardized regression parameters for the mixed model linear regression models at each of the seven regions of interest for the young Australian male and female adults (Sample 1).

Predictors	putamen β p-value	caudate β p-value	pallidum β p-value	hippocampus β p-value	amygdala β p-value	accumbens β p-value	thalamus β p-value
Maximum nicotine use	−0.01 p=0.60	0.02 p=0.39	0.02 p=0.16	−0.01 p=0.59	0.01 p=0.72	0.00 p=0.98	0.03 p=0.07
Maximum alcohol use	−0.05 p=0.58	0.02 p=0.80	−0.13 p=0.17	−0.13 p=0.13	−0.11 p=0.21	−0.02 p=0.78	−0.11 p=0.24
Multi-substance use	−0.04 p=0.16	−0.02 p=0.27	−0.06 p=0.04	−0.07 p=0.01	−0.03 p=0.31	−0.02 p=0.42	−0.01 p=0.68
Maximum cannabis use	0.06 p=0.23	0.03 p=0.54	0.03 p=0.64	0.12 p=0.02	0.10 p=0.07	0.00 p=0.93	0.01 p=0.92

Notes: β = standardized beta coefficients; Bonferroni corrected p-value significance threshold = 0.007; Maximum nicotine use = total lifetime cigarettes ever smoked; Maximum alcohol use = when drinking the most have consumed 4 (female) or 5 (male) or more drinks at least once a week for a month or more; Multi-substance use = total lifetime use of cocaine, amphetamines, inhalants, sedatives, hallucinogens, opioids, ecstasy (including ketamine, GHB or party drugs), non-medical use of over the counter/prescription analgesics, and non-medical use of over the counter/prescription stimulants; Maximum cannabis use = frequency of cannabis use when using the most (never used, once or twice, monthly, weekly, and daily or almost daily).

Table 5.

The number of pairwise observations (upper diagonal), polychoric correlations and (standard errors) in the middle-age US males (Sample 2). Correlations between substance and volumes are shaded.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. Regular cannabis use	474	474	442	474	468	470	474	463	471	473	472
2. Maximum nicotine use	0.23$_{(0.01)}$	475	443	475	469	471	475	464	472	474	473
3. Maximum alcohol use	0.42$_{(0.05)}$	0.25$_{(0.05)}$	443	443	437	439	443	433	440	442	441
4. Multi-substance use	0.85$_{(0.01)}$	0.29$_{(0.06)}$	0.31$_{(0.05)}$	475	469	471	475	464	472	474	473
5. putamen volume	0.00$_{(0.06)}$	−0.12$_{(0.05)}$	−0.06$_{(0.05)}$	−0.02$_{(0.06)}$	469	465	469	458	466	468	467
6. caudate volume	0.06$_{(0.06)}$	0.04$_{(0.05)}$	−0.02$_{(0.05)}$	0.05$_{(0.06)}$	0.31$_{(0.04)}$	471	471	460	468	470	469
7. pallidum volume	0.02$_{(0.06)}$	−0.08$_{(0.05)}$	−0.06$_{(0.05)}$	0.02$_{(0.06)}$	0.49$_{(0.03)}$	0.37$_{(0.04)}$	475	464	472	474	473
8. hippocampus volume	−0.06$_{(0.06)}$	−0.07$_{(0.05)}$	−0.15$_{(0.05)}$	−0.06$_{(0.06)}$	0.29$_{(0.04)}$	0.14$_{(0.05)}$	0.23$_{(0.04)}$	464	464	463	462
9. amygdala volume	0.05$_{(0.06)}$	0.00$_{(0.05)}$	−0.02$_{(0.05)}$	0.05$_{(0.06)}$	0.35$_{(0.04)}$	0.08$_{(0.05)}$	0.27$_{(0.04)}$	0.43$_{(0.04)}$	472	471	470
10. accumbens volume	−0.03$_{(0.06)}$	−0.09$_{(0.05)}$	−0.09$_{(0.05)}$	−0.02$_{(0.06)}$	0.41$_{(0.04)}$	0.15$_{(0.05)}$	0.25$_{(0.04)}$	0.35$_{(0.04)}$	0.55$_{(0.03)}$	474	472
11. thalamus volume	0.06$_{(0.06)}$	−0.15$_{(0.05)}$	−0.05$_{(0.05)}$	0.03$_{(0.06)}$	0.36$_{(0.04)}$	0.11$_{(0.05)}$	0.44$_{(0.04)}$	0.28$_{(0.04)}$	0.28$_{(0.04)}$	0.25$_{(0.04)}$	473

Note: Regular cannabis use = frequency of cannabis use when using the most (never used, once or twice, monthly, weekly, and daily or almost daily); Maximum nicotine use = total lifetime cigarettes ever smoked; Maximum alcohol use = number of days drinking per month when drinking the heaviest; Multi-substance use = total lifetime use of stimulants, sedatives, cocaine, heroin, and PCP or other psychedelics.

Table 6.

Standardized regression parameters for the mixed model linear regression models at each of the seven regions of interest for the middle-aged males (Sample 2).

Predictors	putamen β p-value	caudate β p-value	pallidum β p-value	hippocampus β p-value	amygdala β p-value	accumbens β p-value	thalamus β p-value
Maximum nicotine use	−0.10 p=0.04	−0.01 p=0.77	−0.10 p=0.04	−0.01 p=0.84	−0.01 p=0.84	−0.04 p=0.41	**−0.15 p<0.01**
Maximum alcohol use	−0.03 p=0.88	−0.02 p=0.67	0.04 p=0.42	−0.09 p=0.05	−0.03 p=0.51	−0.06 p=0.19	−0.03 p=0.53
Multi-substance use	−0.02 p=0.96	0.02 p=0.76	−0.02 p=0.79	−0.03 p=0.64	−0.03 p=0.62	−0.01 p=0.85	−0.03 p=0.67
Regular cannabis use	−0.01 p=0.50	−0.02 p=0.66	−0.02 p=0.72	−0.01 p=0.91	−0.02 p=0.70	−0.00 p=0.95	−0.08 p=0.16

Notes: β = standardized beta coefficients; Bonferroni corrected p-value significance threshold = 0.007; Maximum nicotine use = total lifetime cigarettes ever smoked; Maximum alcohol = number of days drinking per month when drinking the heaviest; Multi-substance use = total lifetime use of stimulants, sedatives, cocaine, heroin, and PCP or other psychedelics; Regular cannabis use = lifetime cannabis use (never tried, tried but ≤5 times, tried >5 times).