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The Influence of DAT1, COMT, and BDNF Genetic Polymorphisms on Total and Subregional Hippocampal Volumes in Early Onset Heavy Cannabis Users

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Abstract

Introduction: Hippocampal neuroanatomy is affected by genetic variations in dopaminergic candidate genes and environmental insults, such as early onset of chronic cannabis exposure. Here, we examine how hippocampal total and subregional volumes are affected by cannabis use and functional polymorphisms of dopamine-relevant genes, including the catechol-O-methyltransferase (COMT), dopamine transporter (DAT1), and the brain-derived neurotrophic factor (BDNF) genes.

Material and Methods: We manually traced total hippocampal volumes and automatically segmented hippocampal subregions using high-resolution MRI images, and performed COMT, DAT1, and BDNF genotyping in 59 male Caucasian young adults aged 18–30 years. These included 30 chronic cannabis users with early-onset (regular use at <16 years) and 29 age-, education-, and intelligence-matched controls.

Results: Cannabis use and dopaminergic gene polymorphism had both distinct and interactive effects on the hippocampus. We found emerging alterations of hippocampal total and specific subregional volumes in cannabis users relative to controls (i.e., CA1, CA2/3, and CA4), and associations between cannabis use levels and total and specific subregional volumes. Furthermore, total hippocampal volume and the fissure subregion were affected by cannabis × DAT1 polymorphism (i.e., *9/9R* and in *10/10R* alleles), reflecting high and low levels of dopamine availability.

Conclusion: These findings suggest that cannabis exposure alters the normal relationship between DAT1 polymorphism and the anatomy of total and subregional hippocampal volumes, and that specific hippocampal subregions may be particularly affected.

Keywords: brain-derived neurotrophic factor; cannabis; catechol-O-methyltransferase gene; dopamine transporter gene; hippocampal subfields; hippocampus

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Introduction

Cannabis is regularly used by ~23 million individuals globally.[1] Regular cannabis use may be detrimental to the brain,[2] being associated with neuropsychological, emotional, and motivational impairments.[3,4] The hippocampus in particular may be especially sensitive to the impact of heavy cannabis use, given its high concentration of cannabinoid receptors type 1 (CB_1).[5–7] This is evidenced by neuroimaging results of dose-dependent hippocampal volume reductions in regular cannabis users.[8–11] However, hippocampal alteration has not been consistently observed across studies.[2,12–18] Although it remains unclear why hippocampus volumetric reduction has not been consistently observed in cannabis users, some evidence suggests that noncannabis-specific vulnerability factors including dopamine gene polymorphism may have played a role in this heterogeneity.[19,20] Importantly, dopamine gene polymorphism may also contribute to adverse mental health outcomes in cannabis users.[21–24] Given the rising prevalence of cannabis use, treatment seekers, and the global trends toward decriminalization of cannabis products, it is vital to address the heterogeneity in cannabis use-related effects across users, by examining the contribution of dopamine gene polymorphism and cannabis use on hippocampal morphology.[1]

The neuroanatomy of the hippocampus and hippocampal subregions—subiculum, presubiculum, cornu ammonis (CA) subfields CA1-4, dentate gyrus (DG), and fimbria[25]—is affected by variation in the expression of genes implicated in dopamine regulation.[26] These genes include the catechol-O-methyltransferase (COMT) gene, the dopamine transporter (DAT1) gene, and the brain-derived neurotrophic factor (BDNF) gene: all of which are highly expressed within the hippocampus.[27–31] The COMT gene codes for the enzyme that metabolizes dopamine and may present as one of three variants (i.e., a single nucleotide polymorphism (SNP) at *Val*^{158}*Met*, rs4680): *val/val*, *val/met*, and *met/met*, which are associated with low, medium, and high dopamine availability, respectively.[32] The DAT1 gene, which is involved in dopamine transport and reuptake, consists of a polymorphic 40-base pair (bp) variable number of tandem repeats (VNTR) in an untranslated region, and has common variants including the 9-repeat (*9/9R*) and the 10-repeat allele (*10/10R*) associated with high and low dopamine availability, respectively.[33,34] Lastly, the BDNF gene is not only involved in neuronal plasticity, but also in dopamine receptor expression. It may present with a SNP (*Val*^{66}*Met*) (rs6265), which can lead to a significant reduction in BDNF trafficking to secretory granules and, as a result, to reduced BDNF production in *met* carriers.[35–37]

Human neuroimaging studies show that COMT, DAT1, and BDNF polymorphisms affect the neuroanatomy of the hippocampus[27,28,38–43] and its subregions.[44] These gene variants may further interact with cannabis use to affect cognitive functions ascribed to the hippocampus (e.g., verbal and visuospatial learning).[45–47] Despite the relevance of dopamine gene polymorphism in hippocampal neuroanatomy and cannabis use, only one study to date has examined and revealed the influence of cannabis and COMT polymorphism on brain anatomy in regular cannabis users.[19] It remains unclear whether polymorphisms in genes other than COMT, which are linked to dopamine regulation—such as DAT1 and BDNF—affect the volume of the hippocampus and its subregions in cannabis users.

We aim to address this gap and extend on our previous work[19] by examining the combined effects of cannabis and genetic polymorphism (i.e., DAT1, COMT, and BDNF) on the volume of the hippocampus (quantified using a validated, highly reliable hand-tracing method)[48,49] and its subregions. We hypothesized that reduced hippocampal (total and subregional) volumes would be apparent in cannabis users versus controls,[2] and in those cannabis users carrying variants linked to high relative to low dopamine level (i.e., COMT *met* vs. *val/val*, DAT1 9/9R vs.10/10R, and BDNF *val/val* vs. *met* allele carriers),[41,50] as elevated extracellular dopamine levels may mediate neurotoxic effects. We further explored whether hippocampal volumes would be affected by cannabis use × COMT–DAT1 cross-products.

Materials and Methods

Participants

We recruited 59 male participants from the general community, including 30 cannabis users and 29 controls matched by age, intelligence quotient (IQ), education, symptoms of depression, anxiety, and psychosis (Table 1).

All participants were recruited through web page and flyers, and underwent a comprehensive telephone interview screening on sociodemographic and substance use data. Participants' inclusion criteria were male gender, Caucasian ethnicity, aged 18–30 years, IQ >90, <5 lifetime use of psychoactive substances other than cannabis, nicotine, or alcohol. Cannabis users were included if they started smoking before

Table 1. Sample Sociodemographic, Substance Use Characteristics and Total and Subregional Hippocampus Volumes

	Cannabis users	Controls	t/χ^2, *p*
Demographics			
Age (years)	21.0 (2.3)	22.4 (3.3)	1.84, 0.07
Males (*n* [%])	30 (100)	29 (100)	—
Cannabis use			
Duration (years)	5.7 (2.4)	—	—
Age onset (years)			
First try	15.0 (1.1)	16.7 (2.0)	2.96, <0.05
Regular use	18.1 (2.0)	—	—
Joints			
Daily	2.5 (1.5)	—	—
Past month	86.03 (56.85)	—	—
Past year	987.52 (679.97)	—	—
Lifetime (cumulative)	5203 (4192)	5.1 (11.3)	6.68, <0.01
Alcohol use			
Age of onset (years)	15.0 (1.1)	15.7 (1.5)	2.20, <0.05
Duration (years)	5.7 (2.3)	6.2 (3.1)	ns
Alcohol (units/week)	5.3 (3.8)	3.2 (2.6)	2.35, <0.05
Tobacco use			
Current smokers (*n*)	27 (90.0)	9 (31.0)	21.6, <0.01
Age of onset (years)	16.3 (1.5)	16.3 (2.2)	ns
Duration of use (years)	4.5 (2.7)	4.9 (3.3)	ns
Cigarettes smoked daily (*n*)	6.0 (5.0)	2.4 (5.9)	1.79, 0.08
Hippocampus			
Total volume	4817.6 (482.0)	4919.7 (465.6)	[a]
Subiculum	10700.4 (879.8)	10341.6 (1076.2)	[a]
Presubiculum	7284.6 (549.0)	7246.0 (753.8)	[a]
CA1	5566.9 (569.5)	5243.3 (629.8)	[a]
CA2/3	16852.4 (1653.7)	15595.6 (1779.7)	[a]
CA4/DG	9385.2 (922.8)	8821.4 (1024.0)	[a]
Fimbria	993.2 (219.2)	1123.6 (262.7)	[a]
Fissure	552.5 (193.34)	537.8 (164.0)	[a]
Genetic polymorphism			
BDNF (*n*)			
Met	10	9	ns
Val/val	16	18	
COMT (*n*)			
Met	23	22	ns
Val/val	7	7	
DAT (*n*)			
9/9R	15	13	ns
10/10R	13	16	
COMT × DAT (*n*)			
Val/val + 10/10R	4	3	ns
Met + 10/10R and Val/val +9/9R	10	17	
Met + 9/9R	14	9	
Met + 10/10R and Val/val +9/9R	10	17	
Met + 9/9R	14	9	

For all analyses, df = 58. COMT = *Val*108/158; DAT1 = DAT 3′ UTR VNTR; BDNF = *Val*66*Met*; *Met* = carriers of COMT or BDNF alleles *val/met* and *met/met*.

[a]Results from multiple analyses are outlined in the article body for total hippocampal volume, and Supplementary Table S1 for hippocampal subregions.

CA, cornu ammonis; DG, dentate gyrus; BDNF, brain-derived neurotrophic factor; COMT, catechol-O-methyltransferase; ns, not significant; VNTR, variable number of tandem repeats; UTR, untranslated region.

age 16, if they smoked >14 weekly "joints" for >2 years, and if they tested positive for cannabinoids during the urine toxicology test. Controls were included if they smoked cannabis <15 times, did not smoke in the past month, and if they tested negative for any illicit drugs as ascertained by the urine toxicology test.

Exclusion criteria for all participants were (i) lifetime Axis I, DSM-IV disorder apart from nicotine use disorder, or cannabis use disorder in cannabis users; (ii) positive urine toxicology return for opiates, cocaine, amphetamines, and benzodiazepines; (iii) current psychoactive medications; (iv) history of chronic medical illness or neurological conditions; (v) head trauma with loss of consciousness >2 min; (vi) left-handedness; and (vii) uncorrected visual impairment, color blindness, and hearing impairment.

Procedure

Participants underwent a detailed medical history check, physical/neurological examinations, and urine and hair toxicology tests through immunometric assay kits (Instant-View, ASD, Inc., Poway, CA). The latter corroborated self-reported substance use through the Psychiatric Research Interview for Substance and Mental Disorders (PRISM).[51] Verbal intelligence was assessed with the WAIS-III vocabulary subscale.[52]

All participants provided written informed consent after receiving a complete description of the study, and were financially compensated for any incurred cost. The study was approved by the Ethical and Clinical Research Committee where the study was run (CEIC-Parc de Salut Mar).

Genotyping

The COMT *val*108/158*met* (rs4680) and BDNF *val*66-*met* (rs6265) SNP allelic variants were determined using the 59 exonuclease TaqMan assay with ABI 7900HT Sequence Detection System (real time PCR) supplied by Applied Biosystems. Primers and fluorescent probes were obtained from Applied Biosystems (TaqMan SNP genotyping assays: assay ID C_2255335_10 and C_11592758_10 for rs4680 and rs6265, respectively). Reaction conditions were those described in the ABI PRISM 7900HT user's guide. Endpoint fluorescent signals were detected on the ABI 7900, and the data were analyzed using SDS software, version 2.3 (Applied Biosystems).

DAT1 VNTR genotyping was performed using polymerase chain reaction (PCR).[33] Primers used were Forward 5′-FAM-TGTGGTGTAGGGAACGGCCTGAG

and reverse 5′-CTTCCTGGAGGTCACGGCTCAAGG. Each reaction mixture contained (i) one PCR amplification buffer and 0.3 PCR enhancer solution (Invitrogen, Carlsbad, CA), (ii) 3 mM $MgSO_4$, 200 mM dNTPs, 0.2 mM of each primer, (iii) 1 U of Taq DNA polymerase (Invitrogen), and (iv) 50 ng of genomic DNA as template. Amplification conditions included an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 40 sec at 58°C, 45 sec at 72°C, and 5 min at 72°C. We used a total reaction volume of 10 μL. After PCR, we detected the products of allelic-specific amplifications (allele *9/9R*, 450 bp, and allele *10/10R*, 480 bp) through an automatic ABI 3730XL capillary sequencer. Data were analyzed through GeneMapper Software v3.5 (Applied Biosystems). Sequences for genotyping BDNF are described in detail elsewhere.[53]

After genotype determination, the sample was divided into subgroups based on (i) COMT polymorphism (i.e., *val/val* homozygote and *met*-allele carriers including *val/met* and *met* homozygous); (ii) DAT1 polymorphism (i.e., *9/9R* and *10/10R* allele carriers); (iii) BDNF polymorphism (i.e., *val/val* homozygote and *met*-allele carriers including *val/met* and *met* homozygous); and (iv) combinations of DAT1–COMT variants associated with different dopamine availability levels (Table 1).[54–56] The distribution of COMT, DAT1, BDNF, and COMT–DAT1 genetic polymorphism was in Hardy–Weinberg equilibrium.

MRI data

MRI images were acquired with a 1.5T Signa Excite system (General Electric, Milwaukee, WI) equipped with an eight-channel phased-array head coil. High-resolution T1-weighted anatomical images were obtained by using a three-dimensional fast spoiled gradient inversion-recovery prepared sequence with 130 contiguous slices (TR, 11.8 msec; TE, 4.2 msec; flip angle, 15°; field of view, 30 cm; pixel matrix, 256 × 256; and slice thickness, 1.2 mm).

Images were transferred to a Linux workstation for preprocessing including reorientation, resizing to 1 × 1 × 1 mm resolution, and alignment to the Montréal Neurological Institute standard template (www.fmrib.ox.ac.uk/fsl). The same investigator (A.B.) manually delineated the hippocampus using Analyze software (version 9.0; Mayo Clinic, Rochester, MN), while being blind to group membership. Tracing was performed on coronally displayed MRI slices, from the caudal to the rostral portion of the brain, based on a previously validated protocol.[48,57] Hippocampal boundaries were set, *medially*, by the cerebral spinal fluid (CSF) of the uncal cistern; *laterally*, by the CSF in the temporal horn of the lateral ventricle; and *inferiorly* by the parahippocampal white matter running medially from the temporal horn of the lateral ventricle. We used the protocol developed by Watson et al. to separate the hippocampus from the amygdala.[58]

Intraclass correlation coefficients (absolute agreement) were computed to assess tracing reliability, based on 10 randomly selected images. Intrarater reliability was 0.96 and 0.95, whereas inter-rater reliability against an experienced hippocampus tracer (V.L.) was 0.94 and 0.90, for the right and left hemisphere, respectively. We obtained intracranial volumes (ICVs) through the Voxel Brain Morphometry module (http://dbm.neuro.uni-jena.de/vbm/)[59] of the Statistical Parametric Mapping software (www.fil.ion.ucl.ac.uk/spm/).

To assess hippocampal subregional effect, the hippocampus was also segmented using the FreeSurfer image analysis environment (http://surfer.nmr.mgh.harvard.edu/) version 5.3.0, into several subregions (i.e., fimbria, presubiculum, subiculum, CA1, CA2/3, CA4/DG fields, and hippocampus fissure).[25]

Statistical analyses

We ran a series of four repeated-measures analysis of covariances, with total and subregional hippocampal volumes as dependent variables, hemisphere (i.e., left and right) as repeated measure, and ICV as covariate. Factors in the first three models included COMT genetic polymorphisms (i.e., *val/val* vs. *met*), DAT1 genetic polymorphisms (*9/9R* vs. *10/10R*), and BDNF genetic polymorphisms (*val/val* vs. *met* carriers including mostly *val/met* carriers and three *met/met* carriers), respectively, as well as cannabis use (i.e., user vs. nonuser). Factors in the exploratory model were cannabis use × cross-product of COMT–DAT1 genotypes associated with varying levels of dopamine availability[54]: (i) low dopamine, including carriers of alleles *val/val* and *10/10R*; (ii) high dopamine, including carriers of *met* and *9/9R*; (iii) medium dopamine, including carriers of alleles (a) *val/val* and *9/9R* and (b) *met* and *10/10R* and one cannabis using carrier of the *val/val* and *9/9R*. In addition, we explored the impact of cannabis use as the only factor (without genetic polymorphism as a factor) on hippocampal volumes, using the same model already outlined.

Nicotine use (monthly cigarettes) and alcohol use were not included as covariate in the analyses. Monthly cigarettes were not associated with hippocampal volumes

in cannabis users. Nine controls were tobacco smokers, from whom only two smoked >10 cigarettes per day. Alcohol use did not affect any analyses, at either a significant or trend level, when used as a covariate.

Finally, we ran partial correlations to explore the association between hippocampal total and subregional volumes and cannabis use levels (lifetime, past year, and past month cumulative number of joints) and monthly cigarettes, controlling for ICV. No outlier was observed in hippocampal volume distribution. All analyses were performed using SPSS version 19.0. For all the analyses, the significance threshold was $p < 0.05$.

Discussion

Cannabis use and dopaminergic gene polymorphism were found to have both distinct and interactive effects on total and subregional hippocampal volumes. The left hippocampus volume was nonsignificantly reduced in cannabis users relative to controls ($F_{52} = 3.70$, $p = 0.06$). This trend emerged when cannabis use was the only factor in the model, and dissipated when genetic polymorphism was added as an additional factor (COMT, DAT1, BDNF, and COMT × DAT1 cross-products, see Supplementary Table S1). Interestingly, cannabis-using samples from past studies, with consumption levels similar to those of this study (i.e., mean 15 years age of onset, smoking 22 days/month, 3 years duration), also show nonsignificant hippocampal differences relative to nonusing controls.[12] In contrast, samples of heavier users with longer periods of use (i.e., daily use for >10 years)[10,60] show hippocampal reductions relative to nonusers. Duration of heavy use may have played a role in the discrepancy of these findings, with longer duration of heavy use driving reduced hippocampi.[2,10,12,60,61] This is unsurprising given the direct association between cannabinoid exposure and receptor organization on the hippocampus.[62] A longer duration of heavy cannabis use may be required, before significant hippocampal reduction is observed. Accordingly, in this sample we found an association between smaller total hippocampal volumes and higher cumulative cannabis joints smoked during users' lifetime ($r = -0.41$, $p = 0.046$), past year ($r = -0.35$, $p = 0.08$), and past month ($r = 0.34$, $p = 0.08$), suggesting a dose-dependent relationship between cannabis use and hippocampal volume.

We also found significant effects of cannabis on specific subregions, with a larger volume in CA1 (i.e., in two of the four analyses, which used COMT and DAT1 as factors), CA2/3, and CA4 (i.e., in one analysis using COMT and cannabis as factors) in cannabis users relative to nonusers, when controlling for COMT gene polymorphism and ICV (Supplementary Table S1); this is alongside dose-dependent relationships between the volume of various subregions and cannabis use levels—including larger right CA1 and greater monthly/yearly joints ($r_{53} = 0.29$, $p < 0.05$ and $r_{53} = 0.25$, $p = 0.07$), larger right CA2/3 and greater monthly/yearly/lifetime joints ($r_{53} = 40$, $p < 0.005$, $r_{53} = 0.36$, $p < 0.01$ and $r_{53} = 0.33$, $p < 0.05$), and larger right CA4 and greater monthly, yearly, and lifetime joints ($r_{53} = 0.30$, $p < 0.05$, $r_{53} = 0.26$, $p = 0.06$ and $r_{53} = 0.23$, $p = 0.09$).

Total hippocampal volume and the fissure subregion were affected by cannabis × DAT1 polymorphism (i.e., *9/9R* and in *10/10R* alleles), reflecting high and low levels of dopamine availability ($F_{1,52} = 4.93$, $p = 0.031$, see Fig. 1).

Across all groups, average hippocampal volume was largest in control carriers of the *10/10R allele*,

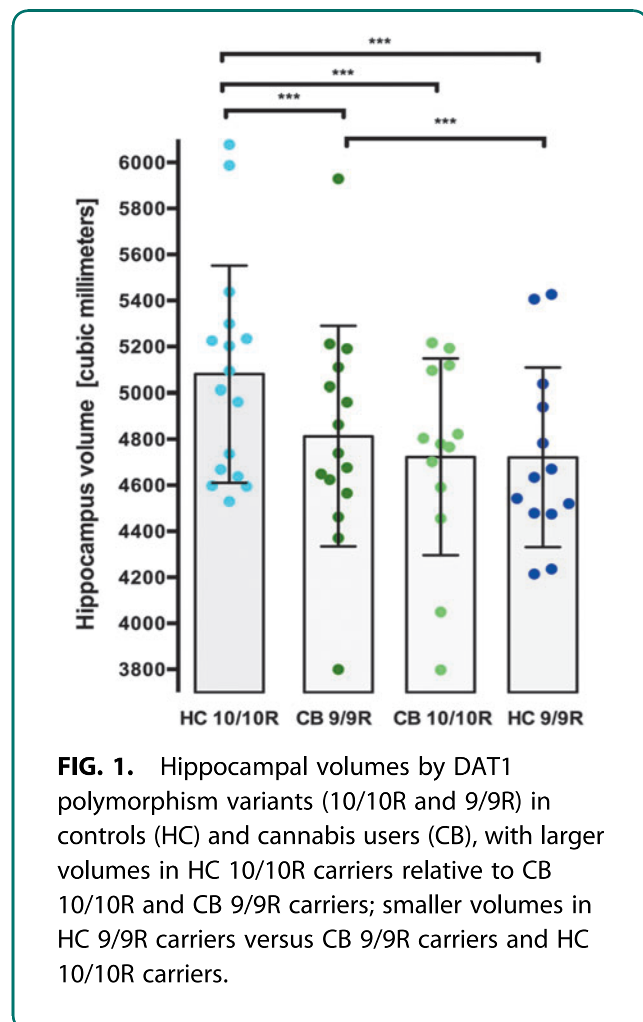

FIG. 1. Hippocampal volumes by DAT1 polymorphism variants (10/10R and 9/9R) in controls (HC) and cannabis users (CB), with larger volumes in HC 10/10R carriers relative to CB 10/10R and CB 9/9R carriers; smaller volumes in HC 9/9R carriers versus CB 9/9R carriers and HC 10/10R carriers.

followed by cannabis-using carriers of the *9/9R* and the *10/10R* allele, and finally by control carriers of the *9/9R* allele. *10/10R*-carrying cannabis users had significantly smaller hippocampi than *10/10R*-carrying controls in the left hemisphere ($t_{27}=7.81$, $p<0.001$, *Cohen'd* $r=-0.8$, large effect size) and right hemisphere ($t_{26}=4.07$, $p<0.0001$; *Cohen'd* $r=-0.7$, large effect size). However, the inverse pattern was observed in *9/9R* carriers, with *9/9R*-carrying cannabis users having larger left hippocampal volumes than *9/9R*-carrying controls ($t_{26}=4.07$, $p<0.0001$, Cohen'd $r=-0.6$, large effect size). This may be related to the DAT1 genotype affecting total hippocampal volumes in controls (i.e., *9/9R* and *10/10R* carriers showed the smallest and largest volumes, respectively—left: $t_{27}=2.69$, $p=0.012$ and right $t_{27}=1.28$, $p=0.21$), but not in cannabis users (i.e., 9/9R and 10/10R carriers had similar hippocampal volumes—*left,* $t_{26}=-0.68$, $p=0.50$ and *right,* $t_{26}=-0.26$, $p=0.79$). Notably, the cannabis × DAT1 effect on total hippocampal volumes may be driven by specific subregion (i.e., hippocampal fissure, Supplementary Table S1) where this effect was also observed. The interaction between dopamine genetic polymorphism (DAT1) and cannabis may partly explain the mixed evidence to date on smaller hippocampal volumes in cannabis users than in controls. This notion remains unexplored, as genetic polymorphism remains underinvestigated in neuroimaging studies of cannabis users.[2]

Our findings suggest that carrying the *9/9R* allele of DAT1 predates smaller hippocampi, and cannabis use may reverse this process. Although the underlying mechanism remains unclear, we speculate that cannabis exposure may selectively interact with DAT1 gene variants to alter the normal effect of dopaminergic transmission on neuroanatomy.[63–65] Chronic cannabis use may alter "optimal" extracellular dopamine levels (e.g., lower synthesis/release and/or contributing to hypersensitivity of postsynaptic receptors)[66] and compromise neuronal growth.[67] The DAT1 gene influences neuronal growth, survival, and differentiation, and is furthermore highly expressed within the hippocampus.[67,68] Importantly, emerging evidence shows that dopaminergic genes (e.g., DAT1)[63,69] mediate the effects of specific cannabinoid compounds on the brain. It remains unclear whether these effects change for distinct cannabinoid compounds that have very different effects on neuroanatomy,[2,70] neural activity,[71] and emotion processing[72]—such as the neuroprotective cannabidiol[73,74] and the psychotogenic tetrahydrocannabinol.[75]

Apart from the interactive effect of cannabis × DAT1 on hippocampal fissure volume, the total and subregional volumes of the hippocampus were not affected by either COMT, DAT1, or BDNF polymorphism alone, nor by cannabis × COMT or cannabis × BDNF interaction. This is consistent with some[38,76,77] but not other findings to date that demonstrated an effect of COMT (i.e., volume reduction in *val* vs. *met* allele carriers)[28,50] and BDNF (i.e., volume reduction in *met* vs. *val* allele carriers).[41] We may have failed to detect an effect of COMT, DAT1, or BDNF polymorphism due to the small sample size, or the relatively lower duration of heavy cannabis use in our user sample. Yet, our findings suggest that neuroanatomy may be affected by a multitude of genetic and environmental (e.g., substance use) factors.

Our third exploratory analysis suggests that the volume of the hippocampus is affected by COMT–DAT1 combinations associated with different levels of dopamine concentration. Hippocampal volumes were (at a trend level) affected by cannabis × COMT–DAT1 cross-products ($F_{2,50}=2.62$, $p=0.08$) and significantly affected by the cross-product of hemisphere × cannabis × COMT–DAT1 ($F_2=3.62$, $p=0.034$). Interestingly, the analysis on COMT–DAT1 products linked to different levels of dopamine availability revealed that the volume of the left hippocampus was the largest within controls, but not cannabis users, who carried alleles linked to high versus low and medium dopamine level. Similarly, other reports show that regional brain volumes (i.e., within the caudate)[78] and activity[45,54,55,79] are affected by the epistasis between dopamine-related genes. We warrant the conduct of studies to examine the role of gene × gene interaction on dopamine availability and neuroanatomy in large normative samples, including cannabis users.[55] Finally, in this model, hippocampal volumes were not affected by cannabis alone ($F_{1,50}=0.82$, $p=0.37$) and COMT–DAT1 cross-products ($F_{2,50}=0.2.29$, $p=0.11$).

Interestingly, we found emerging effects for larger subregional volumes in cannabis users. These effects are surprising given the significant evidence for dose-dependent total hippocampal volume reduction, and subregional volume reduction in cannabis users.[2,10,80,81] This discrepancy may be due to the different method of delineating the total hippocampus (i.e., manual tracing) and its subregions (i.e., automated segmentation). Automated segmentation methods may be more susceptible to magnetic resonance image quality

factors.[82] Our magnetic resonance images presented with artifacts along the temporal lobe, which may have reduced the accuracy of automated segmentations. Alternatively, cannabis may have differential effects in distinct subregions of the hippocampus, given the different distribution of cannabinoid receptors across subregions.[83]

In our study, total hippocampal reduction in cannabis users relative to controls is presented in the left hemisphere, whereas subregional volume enlargement in cannabis users relative to controls is presented in the right hemisphere, which may point to a hemisphere-specific effect as well. Importantly, the finding of larger subregions in cannabis users was inconsistently found across the series of four statistical analyses examining the effect of cannabis and four distinct genetic polymorphisms (COMT, DAT1, BDFN, and COMT × DAT1 cross-products) on the hippocampus. Of the four analyses, larger CA2/3 subregions were detected in only two analyses, and larger CA2 and CA4 were apparent in only one analysis (Supplementary Table S1). The inconsistent findings on larger hippocampal subregions must be interpreted with caution and warrant replication in a separate sample of regular cannabis users.

This study has several limitations. Our small sample size of 59 participants limited the power to detect complex effects of COMT, DAT1, and cannabis. Still, large effect sizes in the cannabis × DAT1 effects on hippocampal volumes (i.e., $r = 0.6$ and $r = 0.8$) warrant replication in large normative cohorts, where the impact of additional genetic polymorphism relevant to neuroanatomy can be measured with adequate power—including cannabis receptor-1 (CNR1)[43] and P2 promoter region (rs2097603).[28] Such work is warranted to unravel the complex gene–environmental interplay that affects neuroanatomical alterations in cannabis users. Using Bonferroni correction for multiple comparison, we used a stringent significance threshold $p < 0.01$ (to control for the four independent variables used to predict hippocampal volumes—COMT, DAT1, BDNF, and COMT–DAT1) and found that only sparse results survived (i.e., cannabis × COMT–DAT1, and cannabis × hemisphere on the fissure).

Furthermore, although we have only considered the effect of cannabis use and genetic polymorphism on hippocampus volume, genetic predisposition may similarly interact with cannabis use to affect the neuroanatomy of other subcortical structures (e.g., ventral striatum),[84] and should be considered in future studies. Finally, we could not discern the impact of nicotine from that of cannabis on hippocampal volumes.[85] Although we found no significant correlation between total hippocampal volumes and monthly cigarettes smoked, we cannot conclusively rule out the effect of nicotine on hippocampal volumes, given the low level of cigarette use in our participant sample (average of <7 cigarettes daily).

Conclusions

Our findings suggest that although cannabis alone may subtly affect hippocampal neuroanatomy, given the trend-level and dose-dependent reduction observed in cannabis users, the interaction between cannabis and DAT1 polymorphism was a stronger predictor of hippocampal volume differences between cannabis users and controls. Hippocampal neuroanatomical differences were observable between DAT1 9/9R and 10/10R-carrying controls but not in cannabis users in our sample, suggesting that cannabinoid exposure may alter the normal relationship between DAT1 polymorphism and hippocampal neuroanatomy. The increasing potency of cannabis, rates of treatment-seeking users, and availability of cannabis products due to recent decriminalization policies warrant the identification of vulnerability factors within cannabis users for adverse neurobiological outcomes, such that targeted prevention and intervention strategies may be developed. We warrant the examination of large-scale adult and adolescent samples from existing consortia collating genetic, neural, and behavioral data (ENIGMA Addiction http://enigma.ini.usc.edu/ongoing/enigma-addiction-working-group/[86] and IMAGEN http://imagen-europe.com/en/consortium.php)[87] to study the role of additional genetic polymorphisms (e.g., CNR1) and nongenetic factors on neuroanatomy (e.g., mental health, socioeconomic status, comorbid substance use, and cannabinoid compounds), and psychosocial outcomes in cannabis users, and to ultimately minimize cannabis-related harms.

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Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all participants for being included in the study.

Author Disclosure Statement

JAC is co-inventor (Mehoulam R, JC, Guimaraes FS, AZ, JH, Breuer A) of the patent "Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023" Def. US no. Reg. 62193296; 29/07/2015; INPI on 19/08/2015 (BR1120150164927). The University of São Paulo has licensed the patent to phytecs Pharm (USP Resolution No. 15.1.130002.1.1). The University of São Paulo has an agreement with Prati-Donaduzzi (Toledo, Brazil) to "develop a pharmaceutical product containing synthetic cannabidiol and prove its safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson's disease, and anxiety disorders". JAC has received travel support from and is medical advisor of BSPG-Pharm. For all other authors, no competing financial interests exist.

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Abbreviations Used

10/10R = 10-repeat allele
BDNF = brain-derived neurotrophic factor
bp = base pair
CA = cornu ammonis
CB_1 = cannabinoid receptors type 1
CNR1 = cannabis receptor-1
COMT = catechol-O-methyltransferase
CSF = cerebral spinal fluid
DAT1 = dopamine transporter
DG = dentate gyrus
ICV = intracranial volume
IQ = intelligence quotient
MRI = magnetic resonance imaging
PCR = polymerase chain reaction
PRISM = Psychiatric Research Interview for Substance and Mental Disorders
SNP = single nucleotide polymorphism
UTR = untranslated region
VNTR = variable number of tandem repeats

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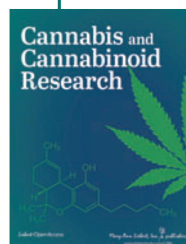

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