2017

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Publication Details

Chye, Y., Suo, C., Yucel, M., den Ouden, L., Solowij, N. & Lorenzetti, V. (2017). Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users. Psychopharmacology, 234 (14), 2149-2157.

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Abstract
Rationale - Cannabis use is associated with neuroanatomical alterations in the hippocampus. While the hippocampus is composed of multiple subregions, their differential vulnerability to cannabis dependence remains unknown.

Objectives - The objective of the study is to investigate gray matter alteration in each of the hippocampal subregions (presubiculum, subiculum, cornu ammonis (CA) subfields CA1-4, and dentate gyrus (DG)) as associated with cannabis use and dependence.

Methods - A total of 35 healthy controls (HC), 22 non-dependent (CB-nondep), and 39 dependent (CB-dep) cannabis users were recruited. We investigated group differences in hippocampal subregion volumes between HC, CB-nondep, and CB-dep users. We further explored the association between CB use variables (age of onset of regular use, monthly use, lifetime use) and hippocampal subregions in CB-nondep and CB-dep users separately.

Results - The CA1, CA2/3, CA4/DG, as well as total hippocampal gray matter were reduced in volume in CB-dep but not in CB-nondep users, relative to HC. The right CA2/3 and CA4/DG volumes were also negatively associated with lifetime cannabis use in CB-dep users.

Conclusions - Our results suggest a regionally and dependence-specific influence of cannabis use on the hippocampus. Hippocampal alteration in cannabis users was specific to the CA and DG regions and confined to dependent users.

Disciplines
Medicine and Health Sciences

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Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users

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ABSTRACT

Rationale Cannabis use is associated with neuroanatomical alterations in the hippocampus. While the hippocampus is composed of multiple subregions, their differential vulnerability to cannabis dependence remains unknown.

Objectives To investigate grey matter alteration in each of the hippocampal subregions (presubiculum, subiculum, cornu ammonis (CA) subfields CA1-4, and dentate gyrus (DG)) as associated with cannabis use and dependence.

Methods A total of 35 healthy controls (HC), 22 non-dependent (CB-nondep), and 39 dependent (CB-dep) cannabis users were recruited. We investigated group differences in hippocampal subregion volumes between HC, CB-nondep, and CB-dep. We further explored the association between CB use variables (age of onset of regular use, monthly use, lifetime use) and hippocampal subregions in CB-nondep and CB-dep users separately.

Results The CA1, CA2/3, CA4/DG, as well as total hippocampal grey matter, were reduced in volume in CB-dep but not in CB-nondep users, relative to HC. The right CA2/3 and CA4/DG volumes were also negatively associated with lifetime cannabis use in CB-dep users.

Conclusions Our results suggest a regionally- and dependence-specific influence of cannabis use on the hippocampus. Hippocampal alteration in cannabis users was specific to the CA and DG regions, and confined to dependent users.

Keywords hippocampus, cannabis, MRI, brain imaging, dependence

INTRODUCTION

Cannabis is widely used for its psychoactive effects, such as alteration of conscious perception and sense of time and space, euphoria, and relaxation; ascribed to the impact of the main psychoactive compound of cannabis, delta^{9}-tetrahydrocannabinol (THC) on the brain cannabinoid receptor type 1 (CB1R) (Gaoni and Mechoulam 1964; Hall and Degenhardt 2007; Svíženská et al. 2008). A global burden of disease study suggested that in 2010, the prevalence of cannabis dependence was estimated at 13 million cases (Degenhardt et al. 2013). Cannabis dependence is associated with substantial psychosocial impairment including interference to productivity, interpersonal relationships difficulties, and poorer emotional and cognitive functioning (Fergusson and Boden 2008; Volkow et al. 2014), raising concern for the associated increase in disability in dependent users (Coffey et al. 2002).

A commonly implicated neuroanatomical region that is altered in cannabis users is the hippocampus (Lorenzetti et al. 2014; Yücel et al. 2016). Preliminary evidence suggests that volumetric reduction of the hippocampus may be driven by a subgroup of users that are dependent on cannabis (Lorenzetti et al. 2016). Dependent users experience a loss of control in their substance use, often using larger amounts or for longer than intended, despite impairment to their social and occupational functioning (American Psychiatric Association 2013). Progression from controlled substance use to such compulsive use is suggested to be characterised by distinct neuroplastic changes to critical brain regions including those involved in reward and conditioned learning, such as the hippocampus (Koob and Volkow 2010; Filbey and Dunlop 2014). However, it is unclear whether this volumetric reduction is subserved by alteration of specific hippocampal subregions (subiculum, presubiculum, cornu ammonis (CA) subfields CA1-4, dentate gyrus (DG), and fimbria) (Van Leemput et al. 2009). This is important because distinct subregions of the hippocampus serve unique functions ranging from learning and memory (Gabrieli et al. 1997), to the regulation of emotional behavior and hypothalamic function (Narr et al. 2004). Consequentially, hippocampal subregions may be differentially affected in disorders involving hippocampal dysfunction, as demonstrated, for example, in Alzheimer's Disease, depression, and schizophrenia (Zhao et al. 2001; Small et al. 2011).

Chronic cannabis use is consistently associated with impaired cognitive functioning along specific domains (attention, verbal learning and memory; rather than working memory) (Broyd et al. 2016), and poorer mental wellbeing, including elevated stress (Degenhardt et al. 2003; Hyman and Sinha 2009). Such impairments are directed by the effect of cannabinoid compounds on the CB1Rs (Sviženská et al. 2008), that are heterogeneously distributed within hippocampal subregions, with a particularly high concentration within the molecular layer of the DG, the strata pyramidale of the CA2 and CA3, and the subiculum (Glass et al. 1997). For example, a rat study demonstrated CB1Rs in the CA1 region to specifically mediate memory impairment in cannabinoid administration (Wise et al. 2009). Meanwhile, chronic stress associated with substance use may disrupt neurogenesis in the DG of the hippocampus (Chambers 2013). Besides region-specific function impairment, evidence in rat studies also demonstrated cannabinoid-induced subregion-specific morphological changes (i.e. upregulation of GABA receptor and reduced dendritic length CA1, dendritic rearrangement in CA3 and lower blade of the DG (Lawston et al. 2000; Verdurand et al. 2010)). Furthermore, human studies have shown alteration to hippocampal shape in cannabis users relative to controls (Solowij et al. 2013; Smith et al. 2015), suggesting morphological differences specific to subregions of the hippocampus. However, no study has yet investigated the relative contributions of various hippocampal subregions to the hippocampal alterations observed in cannabis users, a strategy that may provide insight into the mechanism and implications of these neural effects. Similarly, the extent to which hippocampal subregions may be altered by cannabis use in general, or cannabis dependence specifically remains unknown.

Here, we examine the volume of hippocampal grey matter subregions (presubiculum, subiculum, CA1, CA2 and CA3, CA4 and dentate gyrus (DG), and total grey matter (GM)) and their differential relationships with cannabis use and dependence. We used an automated segmentation method for hippocampal subregions that allows for the generation and quantification of subregional volume (Van Leemput et al. 2009) and has been reliably used in various other studies including major depressive disorders (Han et al. 2016), schizophrenia and bipolar disorder (Haukvik et al. 2015) to discern regionally specific hippocampal alterations. We hypothesise that dependent and non-dependent cannabis users (CB-dep and CB-nondep, respectively) will both demonstrate smaller hippocampal subregion volumes than healthy controls (HC), with additional subregion alteration specific to CB-dep users.

METHODS

Participants

A total of 96 participants, including 35 HC, 22 CB-nondep, and 39 CB-dep age- and gender-matched individuals (reported in previous studies; e.g., Zalesky et al. 2012; Yücel et al. 2016)), were recruited via advertisement in local newspapers and internet websites, and underwent a comprehensive screening to determine eligibility. Inclusion criteria were to be 19 to 55 years of age, right-handed, have an IQ score of >70, to speak English as a first language, and have normal or corrected-to-normal visual acuity. Cannabis users were to have used cannabis regularly (at least twice a month) for at least two years, and be willing to abstain from cannabis use at least 12 hours prior to assessment. Exclusion criteria were any history of neurological disorders or serious head injury; any personal psychiatric history requiring treatment; use of psychoactive medications; significant use of substances besides cannabis, alcohol and tobacco (>50 lifetime occasions); and MRI contraindications. The depression and psychosis modules of the Structured Clinical Interview for DSM-IV-R (SCID) were also administered to exclude participants with any depressive or psychotic disorder (Spitzer et al. 1994; First et al. 2001).

Measures

Participants' demographic and drug use characteristics, including cannabis, alcohol, and tobacco use, were assessed through a semi-structured interview, previously employed in other studies to characterize lifetime and current substance use (Solowij et al. 1995; Yücel et al. 2010; Solowij et al. 2011; Takagi et al. 2013). Participants' self-reported substance use was further corroborated with measure of recent use (Timeline Follow Back, TLFB; Sobell and Sobell, 1992). A urine toxicology test was used to corroborate self-reported cannabis use/nonuse in users and HC, and for detection of illicit substance use, with any discrepancies in reported personal history of use addressed with participants. However, due to the high variability of urinary THC metabolite levels (depending on factors including time of urine sampling, body fat content, and degree of urine dilution (Musshoff and Madea 2006; Lowe et al. 2009)), urinary metabolite levels were not included in our analysis.

CB-nondep and CB-dep users were separated using the Severity of Dependence Scale (SDS), administered as a self-report questionnaire, with a cut-off score of 4 and above

indicating cannabis dependence (Gossop et al. 1995). The SDS has good criterion validity against DSM-IV diagnosis of cannabis dependence (r = .48 – .76), with an optimal differentiating cut-off of 4 and above (sensitivity = 61.3 – 65.1, specificity = 63.5 – 94.3) (Martin et al. 2006; Piontek et al. 2008; van der Pol et al. 2013).

Cannabis use was quantified and converted to a standardised unit of 'cones', based on users' self-report of their amount of use in units familiar to them and use of visual aids (e.g., grams or joints, with 1 gram = 12 cones, and 1 paper joint = 3 cones; https://ncpic.org.au/media/1593/timeline-followback.pdf). The Alcohol Use Disorder Identification Test (AUDIT) was used to assess level of alcohol use and dependence (Saunders et al. 1993). IQ was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999). Global functioning of participants was assessed using the Global Assessment of Functioning scale (GAF) (Hall, 1995). Psychological symptoms (depressive, anxiety and psychotic-like) were assessed with the Beck Depression Inventory (BDI) (Beck et al. 1961), the State and Trait Anxiety Inventory for trait anxiety (STAI-T) (Spielberger 2010), and by means of the weighted (for number of questions answered) total frequency subscale of the Community Assessment of Psychic Experiences (CAPE) (Konings et al. 2006).

MRI data acquisition and processing

All participants were scanned on a 3T Siemens Trio scanner, using a high-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE) sequence (TR = 1,900ms, TE = 2.15ms, field of view = 256mm, voxel size = 0.5 x 0.5 x 1 mm^3). All images underwent noise removal using a pre-filtered rotationally invariant nonlocal means filter (PRINLM) (https://sites.google.com/site/pierrickcoupe/softwares/denoising-for-medical-imaging/mri-denoising) to improve segmentation results (Gaser and Coupé 2010; Eskildsen and Coupe 2011; Manjón et al. 2012). Subsequently, images were processed using the FreeSurfer image analysis environment (http://surfer.nmr.mgh.harvard.edu/) version 5.3.0 to obtain hippocampal subregional volume and intracranial volume (ICV). This process includes motion correction (Reuter et al. 2010), non-uniform intensity (Sled et al. 1998; Zheng et al. 2009), automated talairach transformation, removal of non-brain tissue (Ségonne et al. 2004), segmentation of white matter and grey matter volumes (Fischl et al. 2002), and segmentation of the hippocampus and surrounding areas into several subregions

(i.e., fimbria, presubiculum, subiculum, CA1, CA2/3, and CA4/DG fields, as well as choroid plexus, hippocampal fissure, and inferior lateral ventricle) (Van Leemput et al. 2009). Total hippocampal grey matter (GM) was defined as a sum of presubiculum, subiculum, CA1, CA2/3, and CA4/DG.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics 22.0. A repeated-measures analysis of covariance (ANCOVA) was first conducted to assess the difference in hippocampal subregional volumes between HC, CB-nondep, and CB-dep users, with repeated measures consisting of volumes in the left and right hemisphere. Gender, intracranial volume (ICV), age, IQ, global functioning, depressive, anxiety, and psychotic-like symptoms, tobacco and alcohol use were included as covariates. Further exploration of the association between cannabis use variables and hippocampal subregional volumes was conducted by regression analysis in CB-nondep and CB-dep groups separately, with variables including gender, ICV, IQ, depressive (BDI) and psychotic-like (CAPE weighted frequency) symptoms, monthly alcohol and tobacco use, age of regular cannabis use, quantity of cannabis used per month, and estimated lifetime quantity. Depressive and psychotic-like symptoms, as well as alcohol and tobacco use were selected as predictors for the regression analyses based on previous evidence of association with hippocampal volume (Small et al. 2011; Durazzo et al. 2013; Haukvik et al. 2015; Han et al. 2016; Lee et al. 2016).

RESULTS

Demographic and substance use characteristics are presented in Table 1. IQ, GAF, BDI, STAI-T, CAPE, and monthly tobacco use were significantly different between HC, CB-nondep, and CB-dep groups. We explored correlations between these potential confounding variables and hippocampal subregional volumes in the Supplementary Material 1, to exclude their influence on CB-dependence effect.

TABLE 1 Demographic and Drug Use Characteristics in Healthy Controls (HC), Non-dependent Cannabis Users (CB-nondep), and Dependent Cannabis Users (CB-dep), (Mean/n (SD))

	HC	*CB-nondep* [a]	*CB-dep* [a]	F/X^2	*p*
	N = 35	*N = 22*	*N = 39*		

Age	30.37 (11.46)	36.27 (11.73)	30.38 (9.99)	2.46	.091
Gender (M / F)	18 / 17	11 / 11	18 / 21	0.22	.90
IQ [b]	112.71 (12.97)	104.23 (15.16)	99.69 (11.62)	9.41	< .001**
GAF [c]	86.34 (4.32)	78.27 (9.31)	72.38 (9.08)	29.98	< .001**
BDI [c]	3.40 (5.12)	4.14 (4.18)	12.92 (10.38)	17.24	< .001**
STAI-T [c]	33.37 (7.65)	31.91 (12.16)	45.46 (12.52)	15.73	< .001**
CAPE weighted frequency [c]	1.39 (0.23)	1.48 (0.25)	1.68 (0.30)	11.57	< .001**
Alcohol					
StDr/mth [c]	20.32 (27.40)	19.39 (26.44)	22.28 (26.73)	0.09	.91
AUDIT [c]	9.43 (4.59)	9.16 (5.46)	11.25 (5.39)	1.65	.20
Tobacco					
Smoker status [d]	9 / 26	20 / 2	38 / 1	52.84	< .001**
Onset regular use (years)	17.18 (3.52)	15.52 (3.87)	15.62 (2.92)	1.09	.34
Cig/mth [c]	23.03 (79.93)	312.00 (298.86)	250.60 (187.42)	19.53	< .001**
Cannabis use					
Onset regular use (years)	-	17.23 (3.29)	16.46 (3.44)	0.72	.40
Current use	-	264.32 (246.62)	489.18 (310.20)		
(cones/month)				8.50	.005*
(days/month)	-	21.95 (10.33)	27.46 (4.54)	8.33	.005*
Lifetime use (cones)	-	71,009.68	80,160.33		
		(58,857.07)	(86,280.37)	0.20	.66
THC-COOH (Ng/mg) [e]	-	298.94 (410.93)	922.93 (926.20)	2.55	.014*
Dependence [a]	-	1.50 (0.86)	7.85 (3.26)	79.85	< .001**

[a] Dependence measured by Severity of Dependence Scale (SDS); CB-nondep = score of 3 or less, CB-dep = score of 4 or more (Martin et al. 2006; Piontek et al. 2008; van der Pol et al. 2013).

[b] IQ measured by Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999).

[c] GAF = Global Assessment of Functioning scale (Hall 1995); BDI = Beck Depression Inventory (Beck et al. 1961); STAI-T = State and Trait Anxiety Inventory, trait anxiety (Spielberger 2010); CAPE = Community Assessment of Psychic Experiences (Konings et al. 2006); StDr/mth = number of standard drinks consumed per month; AUDIT = Alcohol Use Disorders Identification Test (Saunders et al. 1993); Cig/mth = number of cigarettes smoked per month.

[d] Smoker status = number of regular smokers compared to non-smokers, ex-smokers, and occasional smokers.

[e] THC-COOH = 11-nor-9-Carboxy-THC, urinary metabolite of delta^9-tetrahydrocannabinol (THC), quantified in nanograms to milligrams.

*P < .05, **P < .001

Repeated-measures ANCOVA between HC, CB-nondep, and CB-dep users, revealed that CB-dep, relative to both HC and CB-nondep users, had a smaller CA1 subregion (p = .050 and p = .017, respectively) and significantly smaller CA2/3 (p = .026 and p = .018), CA4/DG (p = .013 and p = .009) and total hippocampal GM (p = .023 and p = .019) (Table 2 and Figure 1). No significant differences in hippocampal subregional volumes were found between HC and CB-nondep users. There were no gender by group interactions, but females across all groups had smaller volumes in CA2/3 (p = .001) and CA4/DG (p = .015) than males. Tobacco use and IQ were the only covariates that influenced hippocampal subregional volumes. Monthly cigarette use significantly affected the presubiculum ($F_{1,79}$ = 4.78, p = .032) and subiculum ($F_{1,79}$ = 6.97, p = .010) volumes, while IQ influenced the subiculum volume only ($F_{1,79}$ = 4.54, p = .036).

TABLE 2 Hippocampal Volumes by Subregion in Healthy Controls (HC), Non-dependent Cannabis Users (CB-nondep), and Dependent Cannabis Users (CB-dep), (mean (SD), mm^3)

		HC *N = 35*	*CB-nondep* [a] *N = 22*	*CB-dep* [a] *N = 39*	***Group Difference*** [b]	
					$F_{2,87}$	p
Intracranial cavity (10^6)		1.51 (0.15)	1.53 (0.14)	1.56 (0.13)		
Presubiculum	Left	477.41 (65.37)	459.27 (63.49)	453.23 (53.33)	0.76	.47
	Right	449.29 (56.88)	436.80 (43.55)	444.67 (56.31)		
Subiculum	Left	655.58 (71.26)	656.33 (66.92)	637.35 (68.01)	1.60	.21
	Right	651.80 (65.79)	643.08 (49.77)	637.07 (65.21)		
CA1 [c]	Left	331.78 (48.62)	331.45 (32.08)	323.40 (42.76)	3.43	.037*
	Right	353.64 (42.13)	347.70 (25.95)	335.26 (41.23)		
CA2 and CA3 [c]	Left	1,031.38 (107.44)	1,002.18 (116.38)	967.39 (115.92)	3.74	.028*
	Right	1,052.82 (108.93)	1,024.14 (90.17)	981.58 (113.37)		
CA4 and DG [c]	Left	578.81 (52.98)	568.04 (61.33)	541.77 (67.00)	4.63	.013*
	Right	589.78 (57.79)	573.84 (51.25)	551.41 (67.39)		
Total GM [c]	Left	3,424.82 (294.27)	3,374.89 (269.79)	3,268.36 (320.87)	3.81	.026*
	Right	3,454.29 (312.64)	3,386.33 (243.26)	3,312.90 (338.22)		

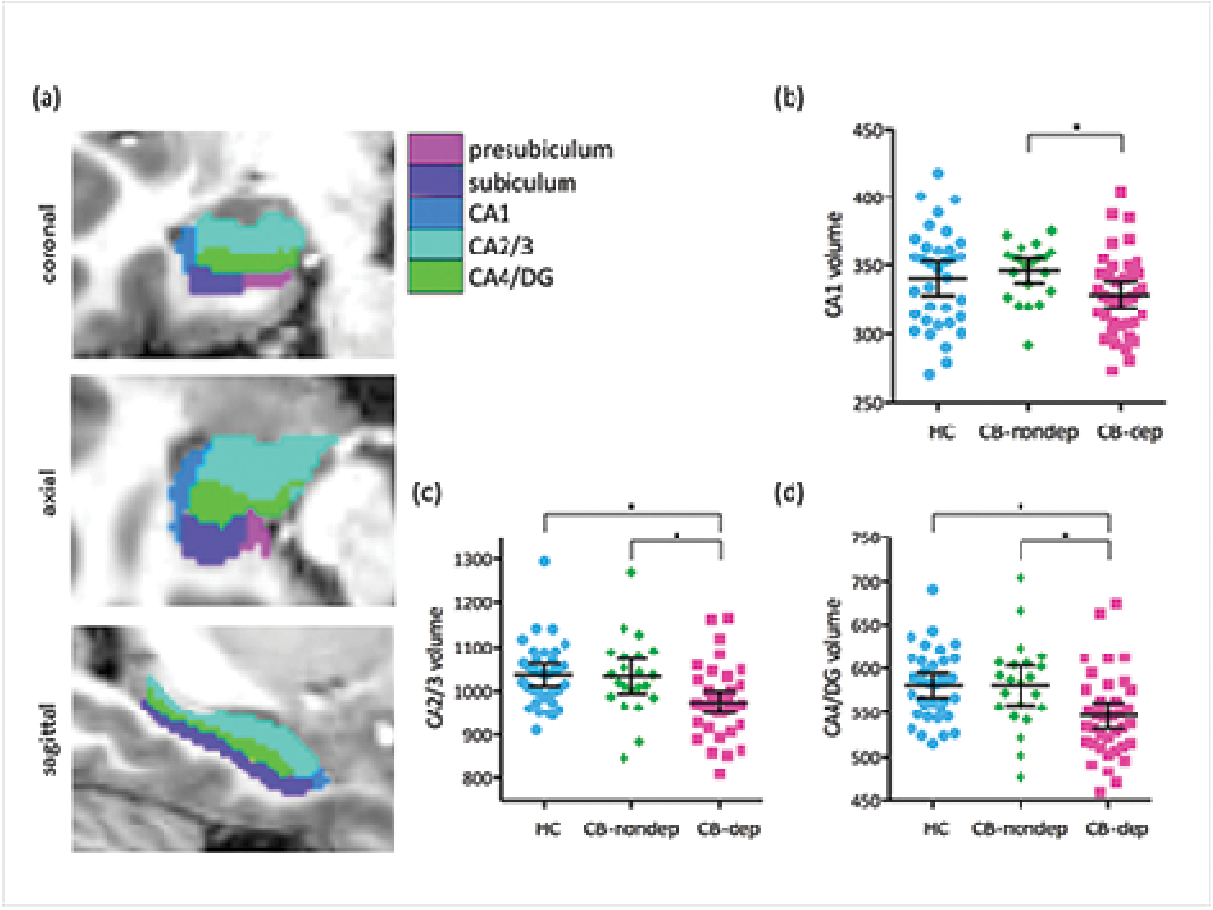
[a] Dependence measured by Severity of Dependence Scale (SDS); CB-nondep = score of 3 or less, CB-dep = score of 4 or more (Martin et al. 2006; Piontek et al. 2008; van der Pol et al. 2013).

[b] F statistic was adjusted for gender, age, IQ, GAF, BDI, STAI-T, CAPE weighted frequency, tobacco use, alcohol use, and total intracranial volume as covariates.

[c] CA = cornu ammonis; DG = dentate gyrus; Total grey matter (GM) volume = sum of presubiculum, subiculum, CA1, CA2 and CA3, and CA4 and DG volumes.

*P < .05

Fig. 1 (a) Cross-sectional slices of an MRI scan with corresponding automated segmentation of hippocampus subfield grey matter; CA = cornu ammonis; DG = dentate gyrus; (b,c,d) CA1, CA2/3, and CA4/DG volumes (collapsed across left and right hemispheres) in controls (HC), non-dependent (CB-nondep), and dependent (CB-dep) cannabis users, adjusted for ICV and gender. Bars represent 95% confidence interval; *p < .05



Further exploration of the association between cannabis use variables and hippocampal subregional volumes was conducted by regression analysis in CB-nondep and CB-dep groups separately, with variables including gender, ICV, IQ, depressive (BDI) and psychotic-like (CAPE) symptoms, alcohol and tobacco use, age of regular cannabis use,

quantity of cannabis used per month, and estimated lifetime quantity. This revealed that lifetime quantity of cannabis use predicted right CA2/3 (*beta* = -.305*, t*(38) = -2.277*, p* = .031), right CA4/DG (*beta* = -.306, *t*(38) = -2.316, *p* = .028), and right total GM (*beta* = -.255, *t*(38) = -2.205, *p* = .036) volumes. Additionally, IQ predicted left CA2/3 (*beta* = .337, *t*(38) = 2.116, *p* = .043), CA4/DG (*beta* = .399, *t*(38) = 2.47, *p* = .020), and total GM (*beta* = .324, *t*(38) = 2.058, *p* = .049) volume in CB-dep users, while tobacco use was not a significant predictor in the models. Meanwhile CB-nondep users showed no significant associations with any variables.

DISCUSSION

Our study is the first that attempts to dissociate the involvement of distinct hippocampal subregions in cannabis use and dependence. We found a reduction in hippocampal CA1, CA2/3, CA4/DG and total hippocampal GM volume confined to CB-dep users relative to both HC and CB-nondep users. HC and CB-nondep users did not differ significantly in hippocampal subregional volumes or total GM. This finding is not only consistent with many other studies in regular cannabis users that have demonstrated hippocampal volume reduction (Lorenzetti et al. 2014; Yücel et al. 2016), but further suggests that reduced volume is driven by or confined to particular subregions and to dependent users. This may account for divergent findings whereby some studies have had less dependent users fail to show reduced hippocampal volume in cannabis users (Lorenzetti et al. 2014; Weiland et al. 2015; Mashhoon et al. 2015). Exploratory regression analysis further explicates cannabis effects on the hippocampus. Reduced right CA2/3 and CA4/DG subregional volumes were associated with lifetime cannabis dosage in dependent users only.

Dependence in CB users indexes out-of-control use, associated with negative mental and physical consequences, rather than quantity of use per se (Piontek et al. 2008). We characterised dependence in our study based on SDS scoring (i.e. with a cut-off of 4 and above as dependent), on a scale of how 'out-of-control' users believe their cannabis use to be, and whether they exhibit difficulties stopping or worry about going without cannabis (Gossop et al. 1995). Our CB-dep and CB-nondep groups differed only in monthly cannabis use, and not in lifetime exposure or age of onset of use. However, current monthly cannabis use did not predict hippocampal subregional volumes in the multiple regression analysis, suggesting recent level of use to have a minimal influence on hippocampal volume. Previous

studies have neglected to consider dependence in examining cannabis-related harms, and as such this dependence-specific effect has gone unnoticed. In future, identifying users with dependent symptoms will be important, in both research and clinical settings, to allow for more targeted intervention. While the SDS is an effective screening instrument against cannabis dependence (Piontek et al. 2008), it should be considered in adjunct to more extensive diagnostic and validating criteria in future, to effectively represent cannabis-related problems in the population.

Our results suggest that cannabis dependence is key in hippocampal subregion volume alterations. Psychopathology symptom scores and global functioning (GAF, BDI, STAI-T, CAPE weighted frequency (Beck et al. 1961; Hall 1995; Konings et al. 2006; Spielberger 2010)) did not influence hippocampal subregional volumes (Supplementary Fig. 1). While IQ was positively associated with left CA2/3 and CA4/DG volumes, suggesting that both greater dependence and lower IQ are associated with smaller hippocampal subregions, it was nevertheless accounted for as covariate in the group analyses, suggesting that the link between cannabis dependence and smaller hippocampal subregions is robust and specific. The specificity of the involvement of CA2/3 and CA4/DG subregions in cannabis dependence is furthermore of importance as analysing specific hippocampal subregions may be a useful means to distinguish the differential involvement of various substances of abuse on hippocampal morphology. For example, a previous study on hippocampal subregion volume in chronic cigarette smokers demonstrated that quantity of cigarettes smoked and tobacco dependence were associated with smaller subiculum and presubiculum (Durazzo et al. 2013). We addressed the influence of tobacco use in our Supplementary Material 1, and only found a weak positive correlation between subiculum volume and monthly tobacco use. Our CB-nondep and CB-dep users were additionally matched by level of tobacco use (p = .33), minimizing its confounding influence on observed dependence-specific CA2/3 and CA4/DG alterations. This observation agrees with a previous study demonstrating no significant hippocampal volumetric difference between cannabis users with minimal tobacco use, and users who used both cannabis and tobacco (Filbey et al. 2015). However, the study demonstrated an effect of 'combined cannabis and tobacco' use on memory performance (Filbey et al. 2015). As such, despite no observable structural differences, future studies should still consider the functional consequences of combined cannabis and tobacco use; as

well as utilise hippocampal subregional analyses as a more fine-grained approach to distinguishing the dependence-specific effects of substances of abuse on the hippocampus.

Our results highlighted a number of moderators to hippocampal alterations. Firstly, the association between lifetime cannabis use and reduced CA2/3 and CA4/DG subregional volumes in the CB-dep group was confined to the right, but not left hemisphere. This may be due to the distinct gene expression pattern in the right and left hippocampus necessary for the functional division of memory processes. For example, the right hippocampus is suggested to be involved in spatial memory, while the left hippocampus is more involved in episodic memory (Burgess et al. 2002). Furthermore, there is a trend towards a right>left hippocampal volume in normal adults (Pedraza et al. 2004), which may result in alterations in the right hippocampus being more apparent. Various other studies have observed hemisphere-specific alterations associated with cannabis use, whether in the hippocampus or other brain regions (Yücel et al. 2008; Demirakca et al. 2011; Cousijn et al. 2012; Smith et al. 2015; Koenders et al. 2016). In sum, the studies highlight the need to consider aberrations in hemispheric asymmetry as a consequence of cannabis dependence. We also noticed gender-related differences, in which CA2/3 and CA4/DG were smaller in females as compared to males. This is unsurprising as sex hormones have been shown to influence CB1R expression (Riebe et al. 2010), and males and females do exhibit different longitudinal trajectories of hippocampal volume change during adolescent neurodevelopment (Suzuki et al. 2005). However, gender differences did not interact with cannabis-dependence-related alterations, and our HC, CB-dep, and CB-nondep groups were well matched by gender, suggesting that gender did not confound the observed dependence-specific alterations.

Our finding of smaller hippocampal volumes specific to the subregions CA1, CA2/3 and CA4/DG in CB-dep users is relevant to understanding the functional impairment in chronic cannabis users. The hippocampal subregions follow a one-way trisynaptic pathway, from the entorhinal cortex, through to the DG, CA3, CA1, and then the subiculum, with each region serving a specific function in the information processing path (Amaral and Witter 1989; Gilbert and Brushfield 2009). The hippocampal subregions CA1, CA3 and DG are critical to support representation, encoding and retrieval of events, allowing the hippocampus to more efficiently organise information and short-term memory (Kesner 2007). Notably, memory impairment is one of the most frequently observed problems associated with heavy

and persistent cannabis use (Broyd et al. 2016), which may be subserved by volume deficits in CA3 and DG specific to chronic dependent cannabis users.

Additionally, the DG is a major site for adult neurogenesis, which is the ability to generate new neurons that integrate into hippocampal neural networks by sending axonal projections to the CA3 (Chambers 2013). This process is implicated in facilitating new memory formation (Eisch 2002; Kempermann et al. 2004), and is suppressed by exposure to addictive substances (e.g. opiates, nicotine, and cocaine) (Chambers 2013). As such, altered DG volume in cannabis-dependent individuals may be mediated by impaired neurogenesis. However, it is important to note that evidence on the effect of cannabinoids on hippocampal neurogenesis has been inconsistent, with findings of reduced, increased, or no change in neurogenesis when cannabinoids are administered in animal studies (Jiang et al. 2005; Kochman et al. 2006; Alén et al. 2010; Schiavon et al. 2016). This may be due to the variety of cannabinoids used in these studies, including synthetic (Jiang et al. 2005; Alén et al. 2010) and non-synthetic (THC (Kochman et al. 2006), and cannabidiol (CBD) (Schiavon et al. 2016)) cannabinoids. Of interest, CBD is suggested to be non-psychogenic, and may be protective against the effect of THC on the brain and hippocampus (Demirakca et al. 2011; Yücel et al. 2016). Further studies are warranted to test the involvement of neurogenesis in cannabis dependence, with particular attention to types of cannabinoid compound, and may inform the development of pro-neurogenic therapies (e.g. exercise (Kandola et al. 2016)) to help treat cannabis dependence.

There are several potential limitations to our study. Firstly, we are unable to partial out dependence-specific alterations that are general across substances, versus those that are specific to cannabis dependence. We did not use the SDS scale to quantify other substance (i.e. tobacco or alcohol) dependence. Meanwhile, alcohol dependence as assessed by AUDIT (Saunders et al. 1993) was well-matched across the group. Given that dependence-specific neuroadaptations may span across different substances (Koob and Volkow 2010), future studies should examine the distinct and interactive effect of various substances in informing dependence-related neuroalterations. Secondly, we acknowledge the possible difference in accuracy of FreeSurfer's automated algorithm in segmenting the different subfields. Van Leemput et al. (2009) demonstrated good dice similarity coefficient (CA2/3 and subiculum = 0.74; CA4/DG and presubiculum = 0.68; CA1 = 0.62) and correlation (CA2/3, r = .91, p ≤ .0002; CA4/DG, r = .83, p ≤ .0028; subiculum, r = .60, p ≤ .066; correlation data

not provided for presubiculum and CA1) between the automated method and gold standard manual segmentation. Nevertheless, the variation in accuracy when segmenting larger structures such as CA2/3 and CA4/DG, versus smaller structures such as CA1, may result in differential sensitivity in detecting changes in these regions. Finally, our study sample was uneven, with 39 CB-dep users and 22 CB-nondep users. While we did not find an association between CB use variables and subregional volumes in the CB-nondep users, the much smaller sample size of the CB-nondep users may have limited our ability to detect a statistically significant effect. Future studies should attempt to reproduce our dependence-related effect in larger samples.

To conclude, our study extends on previous findings of neuroanatomical alteration in the hippocampus in cannabis users to provide a more fine-grained approach to understanding regional- and dependence-specific interactions. We show that hippocampal alteration in cannabis users may be specific to the CA and DG regions, and confined to dependent users. Further studies should explore the functional and behavioral implications of these subregional effects.

COMPLIANCE WITH ETHICAL STANDARDS

This project was funded by the National Health and Medical Research Council of Australia Project Grant (Grant number: 459111). All participants provided written informed content.

FUNDING

M.Y. was supported by a National Health and Medical Research Council of Australia Fellowship (App#1021973) and the David Winston Turner Endowment Fund. N.S was supported by an Australian Research Council Future Fellowship (FT110100752).

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

Alén F, Mouret A, Viveros M-P, et al (2010) Converging action of alcohol consumption and cannabinoid receptor activation on adult hippocampal neurogenesis. Int J Neuropsychopharmacol 13:191–205.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: A review of anatomical data. Neuroscience 31:571–591.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders, fifth edition (DSM-5). American Psychiatric Publishing, Arlington, VA

Beck AT, Ward CH, Mendelson MM, et al (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561–571.

Broyd SJ, van Hell HH, Beale C, et al (2016) Acute and chronic effects of cannabinoids on human cognition—A systematic review. Biol Psychiatry 79:557–567.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35:625–641.

Chambers RA (2013) Adult hippocampal neurogenesis in the pathogenesis of addiction and dual diagnosis disorders. Drug Alcohol Depend 130:1–12.

Coffey C, Carlin JB, Degenhardt L, et al (2002) Cannabis dependence in young adults: An Australian population study. Addiction 97:187–194.

Cousijn J, Wiers RW, Ridderinkhof KR, et al (2012) Grey matter alterations associated with cannabis use : Results of a VBM study in heavy cannabis users and healthy controls. Neuroimage 59:3845–3851.

Degenhardt L, Ferrari AJ, Calabria B, et al (2013) The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: Results from the GBD 2010 study. PLoS One 8:1–14.

Degenhardt L, Hall W, Lynskey M (2003) Exploring the association between cannabis use and depression. Addiction 98:1493–1504.

Demirakca T, Sartorius A, Ende G, et al (2011) Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. Drug Alcohol Depend 114:242–245.

Durazzo TC, Meyerhoff DJ, Nixon SJ (2013) Interactive effects of chronic cigarette smoking and age on hippocampal volumes. Drug Alcohol Depend 133:704–711.

Eisch AJ (2002) Adult neurogenesis: Implications for psychiatry. Prog Brain Res 138:315–342.

Eskildsen S, Coupe P (2011) Effect of non-local means denoising on cortical segmentation accuracy with FACE. In: Organization for Human Brain Mapping 2011 Annual Meeting, Jun 2011, Canada.

Fergusson DM, Boden JM (2008) Cannabis use and later life outcomes. Addiction 103:969–976.

Filbey FM, Dunlop J (2014) Differential reward network functional connectivity in cannabis dependent and non-dependent users. Drug Alcohol Depend 140:101–111.

Filbey FM, Mcqueeny T, Kadamangudi S, et al (2015) Combined effects of marijuana and nicotine on memory performance and hippocampal volume. Behav Brain Res 293:46–53.

First MB, Spitzer RL, Gibbon M, Williams JBW (2001) Structured clinical interview for axis I DSM-IV disorders. New York State Psychiatric Interview, New York

Fischl B, Salat DH, Busa E, et al (2002) Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.

Gabrieli JD, Brewer JB, Desmond JE, Glover GH (1997) Separate neural bases of two fundamental memory processes in the human medial temporal lobe. Science (80-) 276:264–266.

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem … 86:1646–1647.

Gaser C, Coupé P (2010) Impact of non-local means filtering on brain tissue segmentation. Organization for Human Brain Mapping 2010 Annual Meeting, Jun 2010, United States.

Gilbert PE, Brushfield AM (2009) The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. Prog Neuro-Psychopharmacology Biol Psychiatry 33:774–781.

Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77:299–318.

Gossop M, Darke S, Griffiths P, et al (1995) The severity of dependence scale (SDS): Psychometric properties of the SDS in English and Australian samples of heroin, cocaine and amphetamine users. Addiction 90:607–614.

Hall RCW (1995) Global assessment of functioning. A modified scale. Psychosomatics 36:267–275.

Hall W, Degenhardt L (2007) Prevalence and correlates of cannabis use in developed and developing countries. Curr Opin Psychiatry 20:393–397.

Han K-M, Won E, Sim Y, Tae W-S (2016) Hippocampal subfield analysis in medication-naïve female patients with major depressive disorder. J Affect Disord 194:21–29.

Haukvik UK, Westlye LT, Mørch-Johnsen L, et al (2015) In vivo hippocampal subfield volumes in schizophrenia and bipolar disorder. Biol Psychiatry 77:581–588.

Hyman SM, Sinha R (2009) Stress-related factors in cannabis use and misuse: Implications for prevention and treatment. J Subst Abuse Treat 36:400–413.

Jiang W, Zhang Y, Xiao L, et al (2005) Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J Clin Invest 115:3104–3116.

Kandola A, Hendrikse J, Lucassen PJ, Yücel M (2016) Aerobic exercise as a tool to improve hippocampal plasticity and function in humans: Practical implications for mental health treatment. Front Hum Neurosci 10:1–25.

Kempermann G, Jessberger S, Steiner B, Kronenberg G (2004) Milestones of neuronal development in the adult hippocampus. Trends Neurosci 27:447–452.

Kesner RP (2007) A behavioral analysis of dentate gyrus function. In: Progress in Brain Research. pp 567–576

Kochman LJ, dos Santos AA, Fornal CA, Jacobs BL (2006) Despite strong behavioral disruption, delta9-tetrahydrocannabinol does not affect cell proliferation in the adult mouse dentate gyrus. Brain Res 1113:86–93.

Koenders L, Cousijn J, Vingerhoets WAM, et al (2016) Grey matter changes associated with heavy cannabis use: A longitudinal sMRI study. PLoS One 11:1–13.

Konings M, Bak M, Hanssen M, et al (2006) Validity and reliability of the CAPE: A self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand 114:55–61.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238.

Lawston J, Borella A, Robinson JK, Whitaker-Azmitia PM (2000) Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2. Brain Res 877:407–410.

Lee J, Im S-J, Lee S-G, et al (2016) Volume of hippocampal subfields in patients with alcohol

dependence. Psychiatry Res Neuroimaging 258:16–22.

Lorenzetti V, Solowij N, Fornito A, et al (2014) The association between regular cannabis exposure and alterations of human brain morphology: An updated review of the literature. Curr Pharm Des 20:2138–2167.

Lorenzetti V, Solowij N, Suo C, et al (2016) Cannabis dependence and the brain reward system: disentangling the role of exposure and addiction related effects. Eur Neuropsychopharmacol 26:S701–S702.

Lowe RH, Abraham TT, Darwin WD, et al (2009) Extended urinary Δ9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. Drug Alcohol Depend 105:24–32.

Manjón J V., Coupé P, Buades A, et al (2012) New methods for MRI denoising based on sparseness and self-similarity. Med Image Anal 16:18–27.

Martin G, Copeland J, Gates P, Gilmour S (2006) The Severity of Dependence Scale (SDS) in an adolescent population of cannabis users: Reliability, validity and diagnostic cut-off. Drug Alcohol Depend 83:90–93.

Mashhoon Y, Sava S, Sneider JT, et al (2015) Cortical thinness and volume differences associated with marijuana abuse in emerging adults. Drug Alcohol Depend 155:275–283.

Musshoff F, Madea B (2006) Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. Ther Drug Monit 28:155–63.

Narr KL, Thompson PM, Szeszko P, et al (2004) Regional specificity of hippocampal volume reductions in first-episode schizophrenia. Neuroimage 21:1563–1575.

Pedraza O, Bowers D, Gilmore R (2004) Asymmetry of the hippocampus and amygdala in MRI volumetric measurements of normal adults. J Int Neuropsychol Soc 10:664–78.

Piontek D, Kraus L, Klempova D (2008) Short scales to assess cannabis-related problems: a review of psychometric properties. Subst Abuse Treat Prev Policy 3:25.

Reuter M, Rosas HD, Fischl B (2010) Highly accurate inverse consistent registration: A robust approach. Neuroimage 53:1181–1196.

Riebe CJN, Hill MN, Lee TTY, et al (2010) Estrogenic regulation of limbic cannabinoid receptor binding. Psychoneuroendocrinology 35:1265–1269.

Saunders JB, Aasland OG, Babor TF, et al (1993) Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons

with harmful alcohol consumption-II. Addiction 88:791–804.

Schiavon AP, Bonato JM, Milani H, et al (2016) Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice. Prog Neuro-Psychopharmacology Biol Psychiatry 64:27–34.

Ségonne F, Dale AM, Busa E, et al (2004) A hybrid approach to the skull stripping problem in MRI. Neuroimage 22:1060–1075.

Sled JG, Zijdenbos AP, Evans AC (1998) A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans Med Imaging 17:87–97.

Small SA, Schobel SA, Buxton RB, et al (2011) A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat Rev Neurosci 12:585–601.

Smith MJ, Cobia DJ, Reilly JL, et al (2015) Cannabis-related episodic memory deficits and hippocampal morphological differences in healthy individuals and schizophrenia subjects. Hippocampus 10:1–10.

Sobell L, Sobell M (1992) Timeline follow-back: A technique for assessing self-reported alcohol consumption. Meas Alcohol Consum Psychosoc Biochem Methods 41–72.

Solowij N, Jones K a., Rozman ME, et al (2011) Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. Psychopharmacology (Berl) 216:131–144.

Solowij N, Michie PT, Fox AM (1995) Differential impairments of selective attention due to frequency and duration of cannabis use. Biol Psychiatry 37:731–739.

Solowij N, Walterfang M, Lubman DI, et al (2013) Alteration to hippocampal shape in cannabis users with and without schizophrenia. Schizophr Res 143:179–184.

Spielberger CD (2010) State-trait anxiety inventory. In: The Corsini Encyclopedia of Psychology. John Wiley & Sons, Inc.,

Spitzer RL, Williams JBW, Guibbon M, First MG (1994) Structured clinical interview for DSM-IV (SCID). American Psychiatry Press, Washington, DC

Suzuki M, Hagino H, Nohara S, et al (2005) Male-specific volume expansion of the human hippocampus during adolescence. Cereb Cortex 15:187–193.

Svíženská I, Dubový P, Šulcová A (2008) Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures - A short review. Pharmacol Biochem Behav 90:501–511.

Takagi M, Lubman DI, Walterfang M, et al (2013) Corpus callosum size and shape alterations

in adolescent inhalant users. Addict Biol 18:851–854.

van der Pol P, Liebregts N, de Graaf R, et al (2013) Reliability and validity of the severity of dependence scale for detecting cannabis dependence in frequent cannabis users. Int J Methods Psychiatr Res 22:138–143.

Van Leemput K, Bakkour A, Benner T, et al (2009) Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. Hippocampus 19:549–557.

Verdurand M, Dalton VS, Zavitsanou K (2010) GABAA receptor density is altered by cannabinoid treatment in the hippocampus of adult but not adolescent rats. Brain Res 1351:238–245.

Volkow ND, Baler RD, Compton WM, Weiss SRB (2014) Adverse health effects of marijuana use. N Engl J Med 370:2219–2227.

Wechsler D (1999) Wechsler abbreviated scale of intelligence (WASI) manual. Psychological Corporation, San Antonio, TX

Weiland XBJ, Thayer RE, Depue XBE, et al (2015) Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. 35:1505–1512.

Wise LE, Thorpe AJ, Lichtman AH (2009) Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. Neuropsychopharmacology 34:2072–80.

Yücel M, Lorenzetti V, Suo C, et al (2016) Hippocampal harms, protection and recovery following regular cannabis use. Transl Psychiatry 6:e710.

Yücel M, Solowij N, Respondek C, et al (2008) Regional brain abnormalities associated with long-term heavy cannabis use. Arch Gen Psychiatry 65:694–701.

Yücel M, Zalesky A, Takagi MJ, et al (2010) White-matter abnormalities in adolescents with long-term inhalant and cannabis use: A diffusion magnetic resonance imaging study. J Psychiatry Neurosci 35:409–12.

Zalesky A, Solowij N, Yücel M, et al (2012) Effect of long-term cannabis use on axonal fibre connectivity. Brain 135:2245–2255.

Zhao X, Lein ES, He A, et al (2001) Transcriptional profiling reveals strict boundaries between hippocampal subregions. J Comp Neurol 441:187–196.

Zheng W, Chee MWL, Zagorodnov V (2009) NeuroImage improvement of brain segmentation accuracy by optimizing non-uniformity correction using N3. Neuroimage 48:73–83.

Psychopharmacology

Electronic Supplementary Material 1

Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users

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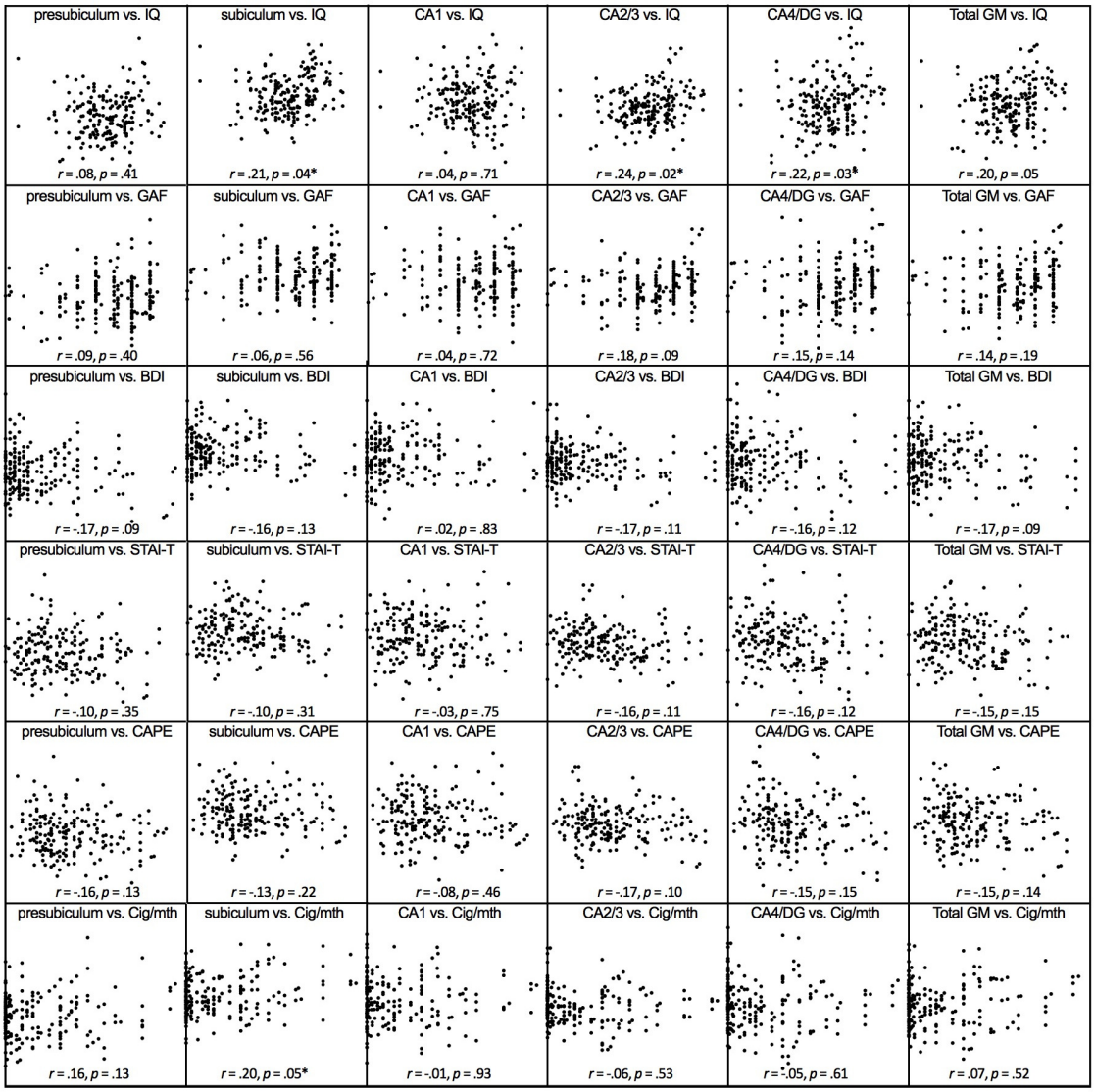
To verify that the observed CB-dep effect was not confounded by other variables (i.e. IQ, global functioning, depressive, anxiety, psychosis symptoms, and monthly tobacco use), we correlated all hippocampal subregional volumes with the aforementioned variables (Supplementary Fig. 1) in the entire sample (N = 96).



SUPPLEMENTARY FIG. 1 Scatterplot of correlation between IQ (Wechsler 1999); global functioning (Global Assessment of Functioning Scale, GAF, Hall 1995); depressive (Beck Depression Inventory, BDI, Beck et al. 1961); anxiety (State and Trait Anxiety Inventory, trait anxiety, STAI-T, Spielberger 2010); psychosis (Community Assessment of Psychic Experiences, CAPE total weighted frequency, Konings et al. 2006) symptoms; tobacco use (number of cigarettes smoked per month) against hippocampal subfield volumes, averaged over left and right hemisphere, corrected for ICV and gender. CA = cornu ammonis; DG = dentate gyrus: GM = sum of presubiculum, subiculum, CA1, CA2, CA3, CA4 and DG; Cig/mth = cigarettes smoked per month. * p < .05

IQ was positively associated with the volumes of the subiculum (r = .21, p = .04), CA2/3 (r = .24, p = .02), and CA4/DG (r = .22, p = .03). However, upon splitting the sample

into HC, CB-nondep, and CB-dep groups respectively, only the correlation between IQ and subiculum volume in the HC group (r = .35, p = .04) remains.

GAF, BDI, STAI-T, and CAPE scores were not correlated with any hippocampal subregional volumes (range of r = -.17 – .18, $p \geq .09$).

Tobacco use (cigarettes per month) was positively correlated with subiculum volume (r = .20, p = .05) in the entire sample. However when the sample was split into HC, CB-nondep, and CB-dep groups, only the association in the HC (r = .40, p = .02) and CB-nondep users (r = .48, p = .03) remain.

Tobacco Use

To further verify that the observed CB-dep effect was not confounded by concurrent tobacco use, we split the CB-nondep and CB-dep users into high and low tobacco use groups respectively, using a cut-off of 200 cigarettes per month (i.e. median monthly cigarette use, see Supplementary Table 1 for group demographics). Repeated-measures ANCOVA, with covariates including gender, ICV, and IQ, determined no effect of tobacco use groups on hippocampal subregional volumes (Supplementary Table 2).

SUPPLEMENTARY TABLE 1 Demographic and Drug Use Characteristics in Non-dependent Cannabis Users with High and Low Tobacco Use (CB-nondep-lowTOB and CB-nondep-highTOB Respectively), and Dependent Cannabis Users with High and Low Tobacco Use (CB-dep-lowTOB and CB-dep-highTOB Respectively), (Mean/n (SD))

	CB-nondep-lowTOB [a,b]	*CB-nondep-highTOB*	*CB-dep-lowTOB*	*CB-dep-highTOB*	F/X^2	*p*
	N = 10	*N = 12*	*N = 20*	*N = 19*		
Age	32.30 (11.83)	39.58 (11.03)	28.25 (9.34)	32.63 (10.41)	2.95	.040*
Gender (M / F)	6 / 4	5 / 7	9 / 11	9 / 10	0.84	0.84
IQ [c]	111.80 (8.36)	97.92 (16.90)	100.16 (13.22)	101.33 (13.07)	2.88	.044*
GAF [d]	82.50 (5.32)	74.75 (10.60)	72.60 (10.54)	72.16 (7.54)	3.36	.025*
BDI [d]	2.10 (1.45)	5.83 (4.97)	15.90 (11.18)	9.79 (8.67)	7.21	< .001**
STAI-T [d]	29.30 (14.75)	34.08 (9.64)	49.80 (12.30)	40.89 (11.33)	8.10	< .001**
CAPE weighted frequency [d]	1.43 (0.18)	1.52 (0.30)	1.78 (0.29)	1.58 (0.28)	4.32	.008*
Alcohol						
StDr/mth [d]	29.09 (33.36)	12.11 (18.09)	22.82 (27.50)	21.72 (26.63)	0.76	.52
AUDIT [d]	10.67 (5.03)	7.89 (5.68)	10.45 (4.66)	12.09 (6.09)	1.49	.23
Tobacco						
Onset regular use (years)	17.67 (3.74)	13.92 (3.23)	16.35 (2.64)	14.84 (3.06)	3.34	.025*
Cig/mth [d]	58.50 (57.10)	523.25 (246.47)	103.65 (64.04)	405.28 (143.45)	35.11	< .001**
Cannabis use						
Onset regular use (years)	19.40 (3.44)	15.42 (1.78)	16.45 (3.14)	16.47 (3.82)	3.05	.036*
Current use (cones/month)	121.20 (119.81)	383.58 (265.02)	485.65 (265.65)	492.89 (358.65)	4.53	.006*
(days/month)	15.50 (10.31)	27.33 (6.87)	27.50 (4.37)	27.42 (4.83)	10.01	< .001**

Lifetime use (cones)	40,546.80 (46,404.49)	96,395.42 (57,488.14)	58,749.80 (58,266.43)	102,697.74 (105,293.23)	2.20	.098
Dependence [a]	1.20 (0.92)	1.75 (0.75)	7.90 (3.66)	7.79 (2.88)	25.90	< .001**

[a] Dependence measured by Severity of Dependence Scale (SDS); CB-nondep = score of 3 or less, CB-dep = score of 4 or more (Martin et al. 2006; Piontek et al. 2008; van der Pol et al. 2013).

[b] lowTOB = low tobacco use = 200 or less cigarettes per month, highTOB = high tobacco use = greater than 200 cigarettes per month.

[c] IQ measured by Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999).

[d] GAF = Global Assessment of Functioning scale (Hall 1995); BDI = Beck Depression Inventory (Beck et al. 1961); STAI-T = State and Trait Anxiety Inventory, trait anxiety (Spielberger 2010); CAPE = Community Assessment of Psychic Experiences (Konings et al. 2006); StDr/mth = number of standard drinks consumed per month; AUDIT = Alcohol Use Disorders Identification Test (Saunders et al. 1993); Cig/mth = number of cigarettes smoked per month.

*$P < .05$, **$P < .001$

SUPPLEMENTARY TABLE 2 Hippocampal Subregional Volumes in Non-dependent Cannabis Users with High and Low Tobacco Use (CB-nondep-lowTOB and CB-nondep-highTOB Respectively), and Dependent Cannabis Users with High and Low Tobacco Use (CB-dep-lowTOB and CB-dep-highTOB Respectively), (mean (SD), mm^3)

		CB-nondep-lowTOB [a,b]	*CB-nondep-highTOB*	*CB-dep-lowTOB*	*CB-dep-highTOB*	***TOB*** [c]		***DEP x TOB*** [c]	
		N = 10	*N = 12*	*N = 20*	*N = 19*	$F_{1,51}$	*p*	$F_{1,51}$	*p*
Intracranial cavity (10^6)		1.54 (0.17)	1.47 (0.11)	1.56 (0.14)	1.55 (0.13)				
Presubiculum	Left	450.11 (75.34)	466.91 (53.96)	452.32 (53.49)	453.78 (53.18)				
	Right	428.23 (53.52)	443.94 (33.99)	451.68 (58.01)	437.67 (53.45)	1.57	.22	2.59	.11
Subiculum	Left	653.35 (60.56)	658.80 (74.40)	641.13 (66.78)	633.42 (69.12)				
	Right	637.43 (59.98)	647.79 (41.63)	635.73 (55.49)	638.71 (74.51)	1.87	.18	1.86	.18
CA1 [d]	Left	339.64 (30.78)	324.62 (50.18)	322.16 (35.07)	323.64 (50.18)				
	Right	345.75 (28.15)	349.32 (25.11)	336.48 (39.94)	333.92 (42.59)	0.10	.75	0.01	.93
CA2 and CA3 [d]	Left	1,035.94 (132.71)	974.04 (97.70)	961.87 (81.76)	967.50 (147.06)				
	Right	1,031.51 (116.38)	1,018.01 (65.95)	979.96 (86.79)	976.64 (140.17)	0.01	.95	0.05	.83
CA4 and DG [d]	Left	582.69 (70.46)	555.84 (52.55)	541.05 (56.22)	540.84 (77.73)				
	Right	576.34 (64.80)	571.76 (39.61)	552.12 (51.84)	546.78 (82.92)	0.00	.97	0.03	.86
Total GM [d]	Left	3,104.07 (1,065.49)	3,341.15 (270.70)	3,262.27 (284.56)	3,260.87 (362.42)				
	Right	3,058.92 (1,061.07)	3,403.52 (173.07)	3,309.76 (275.99)	3,303.38 (400.17)	2.96	.09	2.60	.11

[a] Dependence measured by Severity of Dependence Scale (SDS); CB-nondep = score of 3 or less, CB-dep = score of 4 or more (Martin et al. 2006; Piontek et al. 2008; van der Pol et al. 2013).

[b] lowTOB = low tobacco use = 200 or less cigarettes per month, highTOB = high tobacco use = greater than 200 cigarettes per month.

[c] F statistic was adjusted for gender, IQ, and total intracranial volume as covariates; TOB = fixed effect of tobacco use, DEP x TOB = interaction effect of dependence and tobacco use.

[d] CA = cornu ammonis; DG = dentate gyrus; Total grey matter (GM) volume = sum of presubiculum, subiculum, CA1, CA2 and CA3, and CA4 and DG volumes.

REFERENCES

Beck AT, Ward CH, Mendelson MM, et al (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561–571.

Hall RCW (1995) Global assessment of functioning. A modified scale. Psychosomatics 36:267–275.

Konings M, Bak M, Hanssen M, et al (2006) Validity and reliability of the CAPE: A self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr Scand 114:55–61.

Martin G, Copeland J, Gates P, Gilmour S (2006) The Severity of Dependence Scale (SDS) in an adolescent population of cannabis users: Reliability, validity and diagnostic cut-off. Drug Alcohol Depend 83:90–93.

Piontek D, Kraus L, Klempova D (2008) Short scales to assess cannabis-related problems: a review of psychometric properties. Subst Abuse Treat Prev Policy 3:25.

Saunders JB, Aasland OG, Babor TF, et al (1993) Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. Addiction 88:791–804.

Spielberger CD (2010) State-trait anxiety inventory. In: The Corsini Encyclopedia of Psychology. John Wiley & Sons, Inc.,

van der Pol P, Liebregts N, de Graaf R, et al (2013) Reliability and validity of the severity of dependence scale for detecting cannabis dependence in frequent cannabis users. Int J Methods Psychiatr Res 22:138–143.

Wechsler D (1999) Wechsler abbreviated scale of intelligence (WASI) manual. Psychological Corporation, San Antonio, TX