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Adolescent-onset heavy cannabis use associated with significantly reduced glial but not neuronal markers and glutamate levels in the hippocampus

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Abstract

Cannabis use has been associated with adverse mental health outcomes, the neurochemical underpinnings of which are poorly understood. Although preclinical evidence suggests glutamatergic dysfunction following cannabis exposure in several brain regions including the hippocampus, evidence from human studies have been inconsistent. We investigated the effect of persistent cannabis use on the brain levels of *N*-acetyl aspartate (NAA) and myoinositol, the metabolite markers of neurons and glia, the site of the main central cannabinoid CB1 receptor, and the levels of glutamate, the neurotransmitter directly affected by CB1 modulation. We investigated cannabis users (CUs) who started using during adolescence, the period of greatest vulnerability to cannabis effects and focused on the hippocampus, where type 1 cannabinoid receptors (CBR1) are expressed in high density and have been linked to altered glutamatergic neurotransmission. Twenty-two adolescent-onset CUs and 21 nonusing controls (NU), completed proton magnetic resonance spectroscopy, to measure hippocampal metabolite concentrations. Glutamate, NAA, and myoinositol levels were compared between CU and NU using separate analyses of covariance. CU had significantly lower myoinositol but not glutamate or NAA levels in the hippocampus compared with NU. Myoinositol levels in CU positively correlated with glutamate levels, whereas this association was absent in NU. Altered myoinositol levels may be a marker of glia dysfunction and is consistent with experimental preclinical evidence that cannabinoid-induced glial dysfunction may underlie cannabinoid-induced memory impairments. Future studies using appropriate imaging techniques such as positron emission tomography should investigate whether glial dysfunction associated with cannabis use underlies hippocampal dysfunction and memory impairment in CUs.

KEYWORDS

Cannabis, Glia, H^1MRS, myoinositol, spectroscopy, tetrahydrocannabinol

1 | INTRODUCTION

The long-term use of cannabis, one of the most widely used illicit substances worldwide,[1] has been associated with alterations in a number of cognitive domains.[2-5] Cannabis use, particularly adolescent onset regular cannabis use, has also been associated with increased risk of onset[6,7] and relapse[8-10] of psychosis, as well as greater risk of other adverse mental and behavioural outcomes[11-13] and longer hospitalizations.[14] However, the neurochemical underpinnings of these behavioural, cognitive, and mental health effects associated with persistent cannabis use remain unclear.[15-17]

Delta-9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis, is a partial agonist at the type 1 cannabinoid receptors (CBR1),[18] which are mostly expressed in GABAergic and glutamatergic neurons.[19] Although animal studies have consistently reported evidence of altered dopamine neurotransmission or dopamine levels in several regions,[20-23] this has not been consistently borne out by human studies.[15,24] Investigation of the effects of persistent cannabis use on brain glutamate levels or glutamatergic neurotransmission is also of particular interest. Animal studies have reported dysregulation in glutamatergic synapses of the hippocampus, showing increased[25] as well as decreased glutamate receptor expression[26] and decreased glutamatergic neurotransmission following THC exposure in the hippocampus[26-28] and striatum.[29] In addition, to their predominant neuronal location, recent work has also demonstrated that CB1 receptors are also present on glial cells, particularly astrocytes, which have a role in endocannabinoid-mediated communication between neurons and astrocytes.[30-32] Furthermore, preclinical evidence has also emerged that activation of glial CB1 receptors in the hippocampus by exogenous cannabinoids induces glutamate release and activation of NMDA receptors[31-33] and there is some evidence that astroglial but not neuronal CB1 receptors mediate the memory impairments, which are associated with cannabis use.[34] Given the neuronal and glial localisation of CB1 receptors, the main target of THC and other cannabinoids present in cannabis, one would expect regular cannabis use to affect brain metabolite markers in both neurons (ie, *N*-acetyl aspartate [NAA][35]) and glia (ie, myoinositol[36]) as well as the levels of glutamate, the neurotransmitter directly affected by modulation of the neuronal and glial CB1 receptors. In line with these and other preclinical evidence (summarized in Colizzi et al., 2016), a number of human studies have employed proton magnetic resonance spectroscopy (^{1}H-MRS) to investigate a range of neurochemicals in vivo in cannabis users (CUs). Previous studies of long-term CU have investigated glutamate levels in the basal ganglia,[37,38] frontal and parietal white matter,[37,39] and anterior cingulate cortex.[40,41] These studies have reported decreased glutamate levels in CU compared with nonusing controls(NU) in most[37,39-41] but not all studies.[38] A further recent study investigating a modest sized sample reported no significant difference in hippocampal glutamate levels between CU and NU.[42]

^{1}H-MRS studies have also reported lower levels of myoinositol, an astroglial marker involved in glial function and metabolism,[36] in the thalamus of CU compared with controls, although this was only observed in the left hemisphere.[4] Significantly decreased levels of myoinositol in CU compared with NU have also been observed in the anterior cingulate cortex[41] and in a matrix of voxels including basal ganglia, thalamus, temporal, parietal, and occipital lobe.[43] Other studies have reported nonsignificant changes in myoinositol in the frontal white matter[39] in CU or in the temporal region in cannabis and ecstasy users.[44] Studies investigating NAA, a marker of neuronal integrity,[35,45] have reported decreased levels in CU compared with NU in the prefrontal cortex[46] and hippocampus,[47] and inverse relationship between NAA levels and cannabis use in the inferior frontal gyrus.[44] However, not all previous studies in CU have reported the concentration of all of these metabolites in the brain.

Another pertinent consideration is the potential confounding effects of other drugs, which are often also used by CU. Alcohol and other drugs of abuse may affect glutamate and other metabolite levels in the brain.[48] Among the other common drugs of abuse in regular CUs, cocaine use has been associated with lower levels of glutamate,[49] nicotine dependence has also been associated with changes to the glutamatergic system,[50,51] and alcohol use may affect NAA and myoinositol levels.[52,53]

Therefore, the main objective of the present study was to investigate the effect of persistent cannabis use on the brain levels of NAA and myoinositol, the metabolite markers of neurons and glia, the site of the main central cannabinoid CB1 receptor, and the levels of glutamate, the neurotransmitter directly affected by CB1 modulation. We used ^{1}H-MRS focusing on a brain voxel that included parts of the left hippocampus to compare CU and NU by taking into account the effects of comorbid use of other drugs of abuse. We focused on the hippocampus, as CBR1 are expressed in high density in this region,[54-56] and as animal studies have shown reduced neuronal density in the hippocampus following prolonged exposure to THC[57,58] and modulation of both glial and neuronal CB1 receptors in the hippocampus have been linked to altered glutamatergic neurotransmission.[26-28,32,33] Furthermore, glutamate in the hippocampus is critical for memory encoding,[59,60] which is a key cognitive domains affected by cannabis use.[61,62] We purposely focused on those who had started using cannabis during adolescence because of previous evidence that adolescence is a period when the brain is particularly vulnerable to the consequences of cannabis use.[63,64]

Chronic experimental exposure to high doses of THC has been shown to be associated with significant decrease in astroglial, presynaptic, and postsynaptic but not specific neuronal markers and NMDA receptor levels in the hippocampus of adolescent rats[65] consistent with independent evidence of a specific role of astroglial but not neuronal CB1 receptors in working memory impairment associated with cannabis use.[34] Therefore, we predicted that adolescent-onset regular cannabis use would be associated with selective reduction in astroglial (myoinositol) markers and glutamate levels but not neuronal (NAA) markers in the hippocampus as measured using ^{1}H-MRS in humans.

2 | METHODS

Twenty-two current CU (13 male, 9 female; age 25.05 ± 3.50) and 21, sex and age matched NU (12 male, 9 female; age 24.24 ± 4.11), were

recruited using local and targeted online advertising. All participants provided informed consent and were financially compensated for their time. Ethical approval for the study was obtained from the King's College London Research ethics committee (PNM RESC HR-15/16-2416).

Inclusion criteria required CU to have been consuming cannabis on 4 or more days per week, for the 2 years previous to participating in the study, as assessed by self-report.[66] Information on quantities and types of cannabis used as well as other drug use was also collected[66] (Table 1). CU were required to have started using cannabis regularly, defined as use—at least twice a month,[67] before the age of 18. NU were required to have used cannabis less than 10 times in their lifetime. Exclusion criteria for both groups consisted of a history of neurological disorder, diagnosis of mental illness, or receiving psychiatric treatment; history of psychosis in a first degree relative; an IQ less than 70; and any safety contraindication for magnetic resonance imaging (MRI) scanning.

Participants underwent urine drugs screening (amphetamine, cocaine, opiates, THC, phencyclidine, benzodiazepines, barbiturates, methadone, and propoxyphene) on the day of the MRI visit. NU were required to provide a negative result for all substances, CU were expected to have a positive result for THC, and negative result for all other drugs. Participants were asked to refrain from the use of cannabis or alcohol on the day of MRI scanning and to abstain from caffeine intake for 4 hours and tobacco use within 2 hours of the scan.

2.1 | MRI acquisition

Images were acquired on a General Electric (Milwaukee, Wisconsin) SIGNA HD x 3.0 Tesla system at the Centre for Neuroimaging Sciences at the Institute of Psychiatry, Psychology and Neuroscience, King's College London.

2.1.1 | Structural imaging

Structural images for voxel positioning and calculation of ^{1}H-MRS tissue fractions were acquired using a whole-brain three-dimensional sagittal T1-weighted scan, with parameters based on the Alzheimer's Disease Neuroimaging Initiative (TE = 2.85 ms; TR = 6.98 ms; inversion time = 400 ms; flip angle = 11°; voxel size 1.0×1.0×1.2 mm.

2.1.2 | ^{1}H-MRS

^{1}H-MRS spectra (PRESS - Point RESolved Spectroscopy; TE = 30 ms; TR = 3000 ms; 96 averages) were acquired in a 20×20×15 mm (right-left, anterior-posterior, superior-inferior) voxel positioned in

TABLE 1 – Group descriptives

Group Descriptives and Cannabis Use Parameters	Cannabis Users	Healthy Controls	Statistics
No. Total, n	22	21	
No. Males, n	12	12	Pearson Chi-square= 0.017 *P*=1.0 [a]
Age, mean (SD)	25.05 (3.50)	24.24 (4.11)	*t*= −0.695, Df= 41, *P*=.491
Age [range]	[18-34]	[19-33]	
Years of education, mean (SD)	15.77 (1.96)	16.90 (1.30)	*t*= 2.31, Df= 41, *P*=.026
Life-time joints/episodes of use [range]	[1584-14352]		
Life-time joints/episodes of use average, mean (SD)	4590.86 (4333.15)		
Cannabis age of onset, mean (SD)	14.77 (1.41)		
Cannabis age of onset [range]	[12-18]		
Cannabis years of use, mean (SD)	10.27 (2.12)		
Cannabis years of use [range]	[5-16]		
Current nicotine users, n (%)	12 (54.55%)	4 (19.05%)	Pearson Chi-square= 6.241 *P*=.022[a]
Days of alcohol use in the previous year, mean (SD)	115.82 (90.64)	85.71 (96.57)	*t*= 1.054, Df= 41, *P*=.298
No use of Cocaine in the last year, n (%)	13 (59.1%)	19 (90.48%)	Fisher's exact test statistic= 8.509 *P*=.011[a]
Use of Cocaine at least a few times in the last year, n (%)	9 (40.9%)	2 (9.52%)	
Use of cocaine once or twice a month, n (%)	2 (9.1%)	2 (9.52%)	
No use of MDMA in the last year, n (%)	16 (72.73%)	19 (90.48%)	Fisher's exact test statistic= 5.285 *P*=.104[a]
Use of MDMA at least a few times in the last year, n (%)	6 (27.27%)	2 (9.52%)	
Use of MDMA once or twice a month, n (%)	1 (4.55%)	2 (9.52%)	
No Use of Hallucinogens in the last year, n (%)	18 (81.81%)	21 (100%)	Fisher's exact test statistic= 4.869 *P*=.103[a]
Use of hallucinogens a few times in the last year, n (%)	4 (18.18%)	0 (0%)	
Use of hallucinogens once or twice a month, n (%)	1 (4.55%)	0 (0%)	

[a]Fisher's exact test *P* value.

the left hippocampus (Figure 1), as previously described by Stone et al.[68] The standard GE probe (proton brain examination) sequence was used for acquisition using a chemically selective suppression (CHESS) water suppression. Unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition for each metabolite spectrum. Shimming and water suppression were optimised, with auto-prescan performed twice before each scan.

2.2 | Data analysis

2.2.1 | ^{1}H-MRS quantification

All spectra were analysed with LCModel version 6.3-1L (Provencher, 1993) using a standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myoinositol, L-lactate, N-acetylaspartate, *N*-acetylaspartylglutamate, phosphocholine, and taurine), acquired with the same field strength (3 tesla), localisation sequence (PRESS), and echo time (30 ms). Model metabolites and concentrations used in the basis set are fully detailed in the LCModel manual (http://s-provencher-.com/pages/lcmmanual.shtml). Poorly fitted metabolite peaks (Cramer–Rao minimum variance bounds (CRLB) of >20% as reported by LCModel) were excluded from further analysis. In addition, as recommended by Kreis,[69] we checked whether absolute CRLB values for each metabolite for each participant in the CU group fell within the Mean± 2SD of the CRLB in the control group and excluded from analysis those that fell outside that range. NAA is reported in combination with *N*-acetylaspartylglutamine (NAAG); choline was measured as phosphocholine and glycerophosphocholine (GPC+PCh). Values of the combined water-scaled measure of glutamate, myoinositol, and NAA corrected for cerebral spinal fluid (CSF) content of the region of interest (ROI) using the formula Mcorr = M*(WM+1.21GM+1.55CSF)/(WM+GM), where M is the uncorrected metabolite value and WM, GM, and CSF are the white matter, grey matter, and CSF fractions of the ROI, respectively.[70] These fractions were determined for each subject from the structural T1 scans using Statistical Parametric Mapping 8 software (SPM8), which were used to localise the spectroscopy ROIs and subsequently segmented into grey matter, white matter, and CSF using SPM8.

2.2.2 | ^{1}H-MRS analysis

Data were analysed with Statistical Package for the Social Sciences, version 23 (IBM Corp. IBM SPSS Statistics for Macintosh, Version 23.0. Armonk, NY: IBM Corp.; 2013). Data distribution of glutamate, myoinositol, NAA, and choline was checked for normality. We carried out separate analyses of covariance (ANCOVAs) for glutamate, myoinositol, NAA, and choline as the dependent variables and group (CU vs NU) as the factor. Gender was entered in these analyses as a fixed-effect factor, while age was entered as a covariate, in light of previous evidence suggesting an effect of gender[38,71] and age on metabolite levels.[72-74] Two-tailed correlational analyses with other drugs (calculated as number of occasions used in the 12 months prior to scanning, separately for each drug) were carried out to evaluate any significant association with glutamate, myoinositol, and NAA levels of all participants regardless of group, results showing a trend level significance with cocaine use and glutamate levels. Based on trend level correlations with extent of cocaine use and evidence from previous literature[48,50,51] alcohol, nicotine, and cocaine use in the last year were entered as covariates for the ANCOVA of glutamate levels. For myoinositol and NAA, ANCOVAs were completed with alcohol use in the last year as a covariate based on previous literature.[52,53,75] Age was used as a covariate in all four metabolite analysis as in previous work.[72,76] Additional sensitivity analyses were carried out after excluding users with cocaine and MDMA use in the past year for the key metabolites of interest (glutamate, myoinositol, and NAA). Exploratory correlational analyses examined the association between cannabis use parameters such as age of onset of use, years of use, cost per week, and estimated number of lifetime joints/episodes of use with myoinositol levels in the cannabis-using group in light of altered myoinositol levels in this group.

Association between myoinositol and glutamate levels were assessed separately in the CU and NU groups with one-tailed partial correlational analyses controlling for age, gender, and alcohol use. Correction for multiple testing for correlational analyses carried out was applied within hypotheses (ie, within analyses examining the relationship between metabolite levels [myoinositol and glutamate] and separately within analyses examining the relationship between myoinositol levels and parameters of cannabis use) and not across hypotheses.

3 | RESULTS

Demographics, cannabis, and other drug use parameters for the participant groups are reported in Table 1. All participants in the CU group were regular CU by age 18[67] and were using cannabis on average 6.09 (SD 1.27) days per week at the time of participation in the study.

^{1}H-MRS data from the left hippocampal voxel that included parts of the left hippocampus (please see Figure 1 for the ^{1}H-MRS voxel positioning of the left hippocampus and metabolite spectra) were successfully collected for all 22 CU and 21 NU. CRLBs were less than 20% for all metabolite peaks, and in CU subjects, the absolute CRLB values also satisfied the criterion suggested by Kreis[69]; therefore no subjects were excluded following quality control checks. Data for glutamate, NAA, myoinositol, and choline were found to be normally distributed. There were no significant differences between the groups in grey (P =.075) or white (P=.430) matter and CSF (P=.694) volumes.

Hippocampal glutamate level was found to correlate at a trend level with cocaine use, but not any other drug use, in the last year (r= 0.288; P =.061). No significant correlations were detected between hippocampal myoinositol or NAA and reported use of any other drugs.

3.1 | Hippocampal metabolite levels

All metabolite levels are summarised in Table 2. There were no significant main effects of group (CU vs NU) (P =.676, or gender (P =.140) or any interaction between group (CU vs NU) and gender on

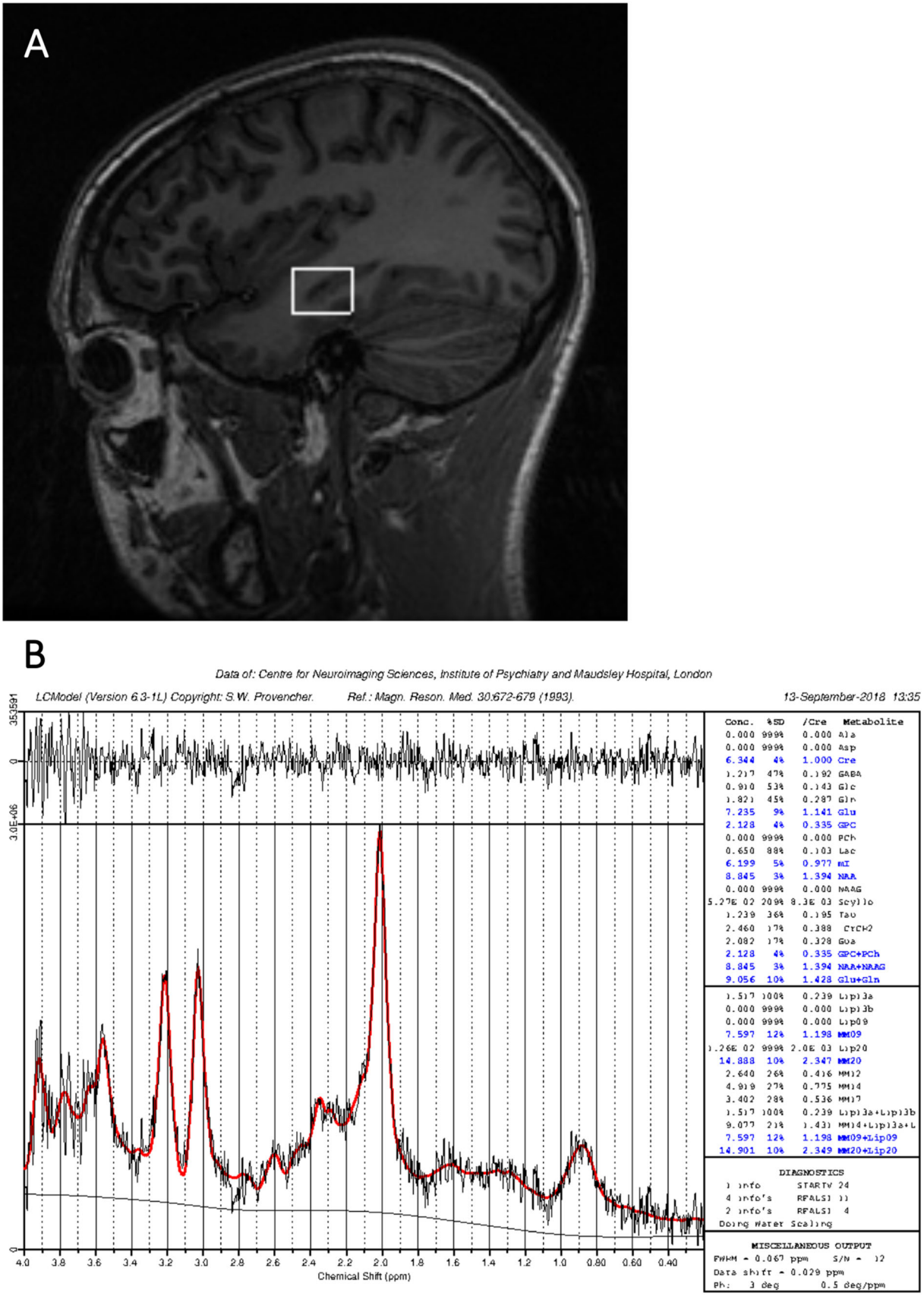

FIGURE 1 – A, Left hippocampal voxel placement for ^{1}H-MRS location. B, Example metabolite spectra obtained for a study participant

TABLE 2 – Metabolite levels and spectra quality

	CU (N=22) (Mean±SD)	NU (N=21) (Mean±SD)	Statistics
SNR	14.00±2.39	12.286±2.57	t=-2.265, Df= 41, p=0.029
FWHM	0.0621±0.011	0.0702±0.0194	t=1.702, Df=41, p=0.096
GM	0.5365±0.192	0.6365±0.058	t=1.83, Df=41, p=0.075
WM	0.2785±0.153	0.3099±0.098	t=0.797, Df=41, p=0.430
CSF	0.0347±0.022	0.0322±0.018	t=-0.396, Df=41, p=0.694
Myoinositol	6.172±0.98 (Male=6.502±0.88; Female=5.695±0.96)	6.761±0.99 (Male=7.023± 0.95; Female=6.413± 0.99)	Group X Gender:$F_{5,37}$ =0.019, p= 0.892 Group:$F_{5,37}$ =6.956, p=0.012, η^2 = 0.158 Gender:$F_{5,37}$ =7.811, p=0.008, η^2= 0.174
NAA + NAAG	9.854±1.06 (Male =10.021±1.06; Female=9.613±1.08)	9.207±1.09 (Male= 9.089±1.22; Female= 9.363±0.93)	Group X Gender:$F_{5,37}$ =0.780, p=0.383 Group:$F_{5,37}$ =2.85, p=0.1 Gender:$F_{5,37}$ =0.044, p=0.836
Choline	2.348±0.34 (Male= 2.43±0.39; Female=2.229±0.22)	2.444±0.302 (Male= 2.467±0.25; Female=2.413±0.38)	Group X Gender:$F_{5,37}$ =0.318, p=0.576 Group:$F_{5,37}$ =1.333, p=0.256 Gender:$F_{5,37}$ =1.694, p=0.201
Creatine	7.329±1.022 (Male= 7.531±1.176) (Female= 7.037±0.711)	7.665±0.784 (Male= 7.853±0.701) (Female= 7.415±0.858)	Group X Gender: $F_{5,37}$=0.159, p= 0.692 Group:$F_{5,37}$ =2.780, p=0.104 Gender:$F_{5,37}$ =3.81, p=0.058

Abbreviations: CSF; cerebrospinal fluid; CU: cannabis using group; FWHM: full width half maximum; GM: grey matter; NAA: *N*-acetyl aspartate; NU: nonusing control group; SNR: signal-to-noise ratio; WM: white matter.

hippocampal glutamate levels after controlling for the effects of age, alcohol, nicotine, and cocaine use.

There was a significant (*P*=.012) main effect of group (CU vs NU) on hippocampal myoinositol levels, such that CU had significantly lower myoinositol compared with NU (Figure 2). There was also a significant main effect of gender (*P*=.008), such that females had significantly lower hippocampal myoinositol (6.054 ± 1.02) compared with males (6.753 ± 0.93). However, the group by gender interaction for myoinositol levels was nonsignificant (*P*=.892). These analyses controlled for the effect of age and alcohol.

There was no significant main effects of group (CU vs NU) (*P* =.100), or gender (*P* =.836) or any interaction between group (CU vs NU) and gender on hippocampal NAA levels after controlling for the effects of age and alcohol.

Further sensitivity analysis carried out excluding subjects with cocaine or MDMA use in the last year (CU=12, NU=18) showed that the general pattern of results remained same (please see Table S1). After excluding subjects with past year cocaine and MDMA use, there was no significant main effect of group (*P*=.159) on left hippocampal glutamate levels after controlling for the effect of age, alcohol, and nicotine. After excluding subjects with past year cocaine and MDMA use, there was no significant main effect of group (*P*=.75) on left hippocampal NAA levels after controlling for the effect of age and alcohol. After excluding subjects with past year cocaine and MDMA use, there was a significant main effect of group (*P*=.012) on left hippocampal glutamate levels after controlling for the effect of age and alcohol.

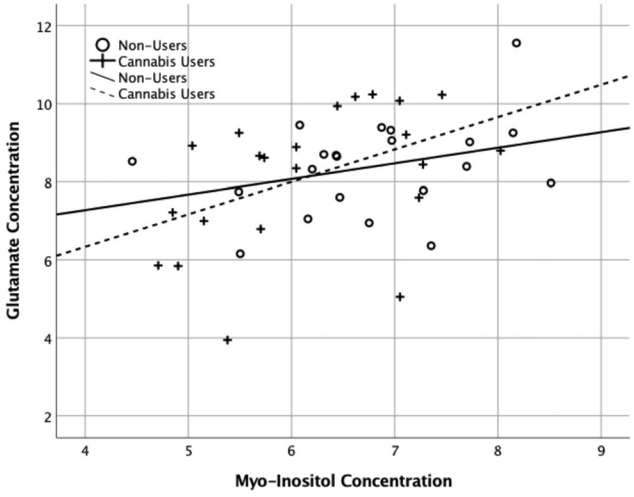

FIGURE 2 – Relationship between left hippocampal glutamate and myoinositol levels

3.2 | Relationship between metabolite levels and parameters of cannabis use

There was no significant relationship between myoinositol levels and parameters of cannabis use such as age of onset, number of years of use, cost per week, and estimated lifetime joints/episodes of use.

3.3 | Metabolite correlations

There was a significant positive correlation between left hippocampal myoinositol and glutamate levels in CU (r= 0.466; P=.022) but not in NU (r= 0.212; P=.106) (Figure 2). Correlation between left hippocampal myoinositol and glutamate levels in CU survived the significance threshold corrected for multiple comparisons (P< .025).

4 | DISCUSSION

The main finding of this study was that compared with nonusers, adolescent-onset regular CUs had significantly lower levels of the glial marker myoinositol in the ^{1}H MRS voxel including parts of the left hippocampus. There were no group differences in left hippocampal NAA (a neuronal integrity marker), or glutamate compared with NU, as measured using ^{1}H MRS. Furthermore, myoinositol levels in CU were positively correlated with glutamate in the hippocampus, whereas no such relationship was found in NU. However, contrary to our prediction, we did not observe a significant difference in glutamate levels in the hippocampus between CU and NU.

Our results are consistent with previous reports of reduced myoinositol levels in the global matrix containing both white and grey matter in the basal ganglia, thalamus, temporal, parietal, and occipital lobe[43] as well as in the thalamus,[77] and anterior cingulate gyrus[41] of CU compared with NU. To our knowledge, our report is the first human study to examine myoinositol levels in the hippocampus specifically, in the context of cannabis use. The results presented here extend previous evidence by showing that adult CU who started using cannabis during adolescence, the period of particular vulnerability to the adverse effects of cannabis, have reduced levels of myoinositol but not NAA or glutamate in the hippocampus, consistent with preclinical evidence of adolescent onset cannabis exposure affecting astroglial markers.[65] These results are also consistent with preclinical evidence of a selective role of astroglia but not neuronal CB1 receptors in memory impairments related to cannabis use.[34]

The lack of significant group difference in hippocampal glutamate levels as measured using ^{1}H-MRS is consistent with a previous report in CU,[42] although other studies have reported glutamate level reduction in other brain regions.[37-41] Increase in striatal glutamate levels have been reported following acute THC exposure in previous studies.[78,79] As CU participants in our study tested positive for THC on urine drug screening on the study day, it is possible that residual THC present in their brain may have affected our ability to detect significant difference in hippocampal glutamate levels between CU and NU. However, residual THC may not necessarily affect the detection of significant alteration in glutamate levels in brain regions as a significant reduction in striatal glutamate levels has also been reported in long-term CU compared with non-users, when they have been studied without a washout period.[37,38] It is also worth noting that in a previous study, while striatal glutamate levels were increased following acute THC exposure, the same effect was not evident in the hippocampus.[79] Therefore, it seems unlikely that the lack of significant group difference in hippocampal glutamate levels in our study was a result of residual THC present in CU. Nevertheless, we cannot completely rule out this possibility. Our results as well as the study by van de Giessen and colleagues suggest that this reduction may be absent in the hippocampus. These differences are not easily explained by differences in sample size or other experimental methodology. However, as our study only investigated the hippocampus, we cannot compare directly with metabolite levels in other regions within this cohort.

Altered myoinositol levels in the hippocampus of CU may be a marker of astroglial dysfunction that may in turn result in altered hippocampal function and hippocampally-mediated cognitive impairments associated with cannabis use. Glial cells are involved in inflammatory and homeostatic regulation, modulation of neurotransmission, and signal transducers, and, especially relevant to the present study, in the modulation of glutamate metabolism.[80] The correlation in CU between myoinositol and glutamate levels may be suggestive of glia dysfunction contributing to lower levels of glutamate availability, a relationship not seen in NU. Astrocytes, following stimulation from glutamate, can increase neurotransmitter release, neuronal activity, and glial transmitter levels.[81] Consequently, this can lead to increased long- and short-term potentiation. Most glutamatergic synapses are in contact with astrocytic processes, although synaptic leakage occurs in the hippocampus at synapses where there are no astrocytes present.[82] Synaptic strength is regulated by the efficacy of astrocytic glutamate uptake[83]; therefore, slower glutamate clearance may result in the activation of presynaptic metabotropic glutamate receptors and inhibition of transmitter release.[84] However, it is worth noting that the association between left hippocampal myoinositol and glutamate levels in CU was observed in the absence of a main effect of cannabis use on hippocampal glutamate levels. Therefore, this association should be considered preliminary and needs independent confirmation in larger samples.

Impaired synaptic function induced by repeated THC exposure has been shown to be associated with reduced uptake of glutamate by glutamate transporters in astrocytes in animal studies.[85] THC activates CB1 receptors on astrocytes that release glutamate in to the synapse, and this is associated with internalisation of glutamate receptors on the post-synaptic membrane,[34] resulting in sustained elevation and accumulation of extracellular glutamate. Consistent with this, repeated exposure to exogenous cannabinoids has been found to reduce NMDA receptor levels in the hippocampus of adult rats exposed to cannabis during adolescence,[65] and reduction in the density of dendritic spines in hippocampal neurons, leading to deficits in long-term synaptic plasticity.[85] Therefore, the effects of cannabis exposure on brain glutamate levels are likely to be complex, with potentially opposing patterns of effects at the astrocytic and the postsynaptic level. However, ^{1}H-MRS as employed here does not allow delineation of these opposing effects or the investigation of changes in receptor levels, which warrant examination in future studies employing positron-emission tomography (PET) imaging using appropriate radiotracers. PET imaging will also allow a more direct investigation of the effect of cannabis use on glial function to confirm results presented here.

What might this mean in terms of behavioural and mental disorders associated with cannabis use? Preclinical evidence suggests that dysfunction in astroglia following cannabinoid exposure is associated with memory deficits,[34,65] one of the most common behavioural effects of cannabis use.[3,86] Reductions in myoinositol, as well as glutamate levels, have been found in the hippocampus of patients with schizophrenia.[87] Reductions in astrocyte numbers have also been reported in people with schizophrenia.[88-90] This may suggest points of convergence in terms of abnormalities present in those with schizophrenia and those with adolescent-onset cannabis use, which has been linked in a dose-dependent manner with increased risk of onset[6,7,91] and relapse[8,92] of schizophrenia. However, it is worth noting that participants in our CU group were all otherwise healthy with no history of psychiatric illness, limiting us from drawing conclusion with our observations and the development of adverse mental disorders. Longitudinal studies in larger cohorts may be able to assess changes in myoinositol and glutamate levels in CUs and relate those changes to development of a psychiatric disorder.

A number of limitations of the present study warrant discussion. Our study focused only on metabolites in the hippocampus in light of its critical role in memory function, consistently shown to be affected by persistent cannabis use. However, we did not investigate a memory task outside the scanner, which would have helped clarify the functional significance of our results. Future studies therefore need to relate brain metabolite alterations in the context of cannabis use with potential functional consequences. It is also worth noting that the sample studied here was relatively modest in size. Nevertheless, we were able to detect significant group differences in hippocampal myoinositol levels, but not in glutamate or NAA. Therefore, future studies need to investigate these in larger samples as well as in other brain regions of interest (eg, basal ganglia and prefrontal cortex) to provide independent confirmation. It is worth noting that ^{1}H MRS hippocampal spectra may have low resolution and poor signal quality due to proximity to sinuses and small size of the region. In our study, signal-to-noise ratio was significantly different between CU and NU. While this may have affected our ability to detect significant group differences in glutamate levels, it is also worth noting that we were nevertheless able to detect significant group differences in myoinositol levels. However, we cannot completely rule out this possibility. There is a possibility that glutamate levels in women may be more sensitive to cannabis use after looking at the pattern of our results and others.[38] Future studies in larger cohorts should therefore also investigate potential effects of gender on brain metabolite levels in the context of cannabis use. Comorbid drug use, particularly in the CU group, may also limit the findings of this study, although we aimed to control for these effects in our analyses. A previous study in a cohort of CUs with no reported other drug use found comparable results as in the present study.[43] Further, sensitivity analysis carried out after excluding subjects with a history of cocaine or MDMA use in the last year showed that the general pattern of group differences or lack thereof in metabolite levels remained unchanged. It is also possible that controlling for numerous covariates such as other drug use may have affected our ability to detect significant alterations in metabolite levels specifically associated with cannabis use. Studies in those who use only cannabis may be necessary to definitively address this issue. However, those who use alcohol and substances of abuse do not necessarily use one substance alone and CUs such as those studied here are perhaps more representative of the population of CUs more generally. It is also worth noting that we employed a cross-sectional design, thereby limiting our ability to disentangle the nature of the relationship between cannabis use and changes in myoinositol reported here, whether cannabis use is a cause or a consequence of these alterations. Longitudinal and/or genetically informed designs (such as twin or sibling samples)[93] are necessary to understand the nature of the relationship. Another important methodological issue that needs consideration in future studies involves the investigation of CU after a period of abstinence. This will help understand whether changes in brain chemistry or lack thereof, associated with cannabis use may be confounded by residual THC present in the system, as evident from comparable studies of effects of cannabis use on brain function.[94,95]

To summarize, compared with NU, CUs were found to have lower levels of the putative astroglial marker myoinositol but not significantly different levels of glutamate or neuronal integrity marker NAA in the voxel including parts of the left hippocampus in CUs compared with NU, consistent with preclinical evidence. Future studies need to employ more direct measures of glial function, such as employing PET imaging, and longitudinal design to confirm these results and relate them to functional, behavioural, and symptomatic consequences of cannabis use.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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