**Cannabis Use is Associated with Lower Hippocampus and Amygdala Volume:
High-Resolution Segmentation of Structural Subfields in a Large Non-clinical Sample**

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ABSTRACT

There is mixed evidence that individuals who use cannabis have reduced hippocampal and amygdalar gray matter volume, potentially because of small sample sizes and imprecise morphological characterization. New automated segmentation procedures have improved the measurement of these structures and allow better examination of their subfields, which have been linked to distinct aspects of memory and emotion. The current study applies this new segmentation procedure to the Human Connectome Project Young Adult dataset ($N = 1081$) to investigate associations of cannabis use with gray matter volume in the hippocampus and amygdala. Results revealed significant bilateral inverse associations of hippocampal volume with recent cannabis use (positive THC urine drug screen) and total lifetime consumption ($p < .001$), as well as association of the left amygdala total lifetime consumption ($p < .001$). Hippocampal subfield analyses indicated these associations were primarily driven by the head of the hippocampus, the first section of the cornu amonis (CA1), and the subicular complex, which have been linked to verbal working memory, episodic memory, and spatial memory respectively. In the amygdala, the association was driven by subfields in the basolateral complex, which links the amygdala to the cortex and is critical in emotion regulation. No associations were detected for age of cannabis initiation or lifetime presence of cannabis use disorder. In one of the largest studies to date, these results support the hypothesis that cannabis use is linked with hippocampal and amygdalar volume and clarify both the specific subfields and the specific aspects of cannabis use that are responsible for these links.

INTRODUCTION

Cannabis is one of the most widely used psychoactive substances worldwide¹ and is increasingly available in legal markets (e.g., Canada, some U.S. states). Because of this, it is important that the effects of cannabis on the brain be fully understood and the literature on these effects be robust and replicable. Furthermore, research suggests that, as cannabis has increased in potency (i.e., increasing content of tetrahydrocannabinol [THC] and decreasing content of cannabidiol [CBD])², that the effects of cannabis on the brain structure may be increasing as well³. One effect of cannabis on the brain that has been repeatedly demonstrated is lower levels of gray matter volume in the hippocampus in cannabis users⁴. This has been found across in contexts and populations, albeit typically in very heavy users in small samples⁵⁻⁷. Additionally, a larger study ($N = 111$) has shown that differences in in hippocampal gray matter volume in cannabis users do not extend to former users, suggesting that there may be changes to hippocampal volume caused by cannabis which reverse over time⁸. However, these findings of reduced hippocampal volume in cannabis users are not uniform across the literature, as a recent mega-analysis of 185 cannabis dependent individuals and 246 controls failed to find differences in hippocampal or amygdalar volume⁹, as did a 2017 study of 20 young adult heavy cannabis users and 23 matched non-cannabis using healthy controls¹⁰.

However, the hippocampus is not a homogenous structure and new research has begun to investigate the effects of cannabis use on specific subfields of the hippocampus. Anterior-to-posterior the hippocampus is divided into its head, body, and tail¹¹. Throughout these three divisions, the hippocampus consists of several histologically differing subfields that are interconnected through a number of pathways that pass information in and out of the hippocampus through the entorhinal cortex, and among the subfields of the hippocampus^{11,12}. The

hippocampal subfields have been shown to fulfill different roles in the memory processes conducted in the hippocampus; for example, the dentate gyrus and third section of the cornu ammonis (CA3) having been found to be involved in memory encoding¹³ and early retrieval, while the first section of the cornu ammonis (CA1) has been linked with episodic memory¹⁴, memory consolidation, late retrieval, and recognition^{15–19}. In the first study to examine the association of cannabis with hippocampal subfields, Chye et al.²⁰ used Freesurfer's automated segmentation software (<https://surfer.nmr.mgh.harvard.edu>) to find many subfields of the hippocampus differentiated 39 cannabis dependent individuals from 35 non-users: CA1, CA2, CA3, CA4 and the dentate gyrus (DG). This study also found the CA2, CA3, CA4, and DG to be associated with lifetime amount of cannabis used. Another study in 89 young adults found similar results, identifying differences between cannabis users and controls in the CA1, CA2/3, and CA4 as mapped by Freesurfer. However, since that time, due in part to concerns about hippocampal subfield segmentation procedures in prior version of Freesurfer²², new subfield segmentation programs for the hippocampus and amygdala have been released in Freesurfer version 6.0^{23,24}, which significantly improved the resolution and accuracy of hippocampal and amygdalar subfield segmentation, while also allowing the ability to quantify the volume of several smaller structures in the hippocampus including the parasubiculum, HATA, fimbria, and hippocampal fissure. The new hippocampus segmentation was used by Beale et al.²⁵ to investigate changes in hippocampal subfield volume in 20 heavy cannabis users who received CBD for 10-weeks. However, this study did not compare these cannabis users to healthy controls, limiting the ability of this study to make inferences about the effects of cannabis on the hippocampal subfields identified by Freesurfer 6.0.

There have also been a number of studies linking cannabis use to smaller amygdalar volume^{26–28}, but, similarly, the amygdala is not one homogenous brain structure, but rather composed of subfields that are unique in cytoarchitecture and histology²⁹. The amygdala's subfields are typically grouped into the basolateral complex, made up of the lateral nucleus, basal nucleus, accessory-basal nucleus, paralaminar nucleus, and corticoamygdaloid transition; and the corticomедial nuclei made up of the cortical nucleus, central nucleus, medial nucleus, and the anterior amygdaloid area^{29,30}. These subfields are interconnected through various pathways which are responsible for emotional processes such as fear conditioning, reward processing, aggression, and addictive behavior³⁰. The best understood of these pathways is for fear conditioning, which occurs via a pathway entering into the lateral nucleus, then connecting to the basal nucleus, then to the central nucleus, and then out to other subcortical structures³⁰. Other specific nuclei have been linked with reward, motivation, and addiction³⁰. There has not been, to our knowledge, any published research on the association of cannabis with the volume of these amygdalar subfields.

Along with a general lack of research on the subfields of the amygdala and hippocampus in cannabis users, another limitation of the existing literature is a major focus on individuals with very heavy cannabis use rather than those using in a way that is more typical in the population. One exception includes a study by Chye et al. that examined 22 non-dependent cannabis users, finding them not to differ from healthy controls in hippocampal volume²⁰. Additionally, the largest investigation of cannabis use in a non-clinical sample to date was a two-part study, with study one including 622 young adults and study two including 474 middle-aged men drawn from the general population; this investigation also did not find associations between hippocampal or amygdalar volume and cannabis use in either study after correcting for multiple comparisons³¹. However, one concern in concluding that there are no differences in hippocampal or amygdalar

volume in recreational cannabis users from this study is that the study included only a coarse single-item measure of cannabis use, “*Have you ever used cannabis once a week or more?*” which allows for the possibility that important features of cannabis use were overlooked, such as heaviness of use, age of initiation, and recency of use. Additionally, data from this study were analyzed using an older version of Freesurfer (v3.0) and were collected with a 1.5-Tesla MRI scanner, both of which may have resulted in less sensitivity to detect effects than the current study which used the most advanced data acquisition and analysis approaches available.

Thus, the aim of the current study was to investigate which hippocampal and amygdalar subfields are most associated with cannabis use using more nuanced measures of cannabis use, the improved Freesurfer 6.0 segmentation procedures, and data from a 3-Tesla MRI scanner. This was done by utilizing data from the Young Adult Human Connectome Project (HCP) to test these associations in one of the largest studies to date. To determine if specific cannabis use features are most related to hippocampal and amygdalar volume, the current study examined four cannabis use variables: total lifetime consumption, , age of initiation, lifetime cannabis use disorder status, and recent use, defined by the presence of THC in a urine drug screen administered on the day of the scan.

METHODS

Participant Characteristics

MRI data were collected from 1113 participants at Washington University in St. Louis over the course of two days as part of the Human Connectome Project between August 2012 and October 2015, and released in full on March 1, 2017. Informed consent was obtained for all participants. Participants were 22-35 years old and had no significant history of psychiatric disorder, substance abuse, neurological disorder or damage, cardiovascular disease, or Mendelian

genetic disease (e.g., cystic fibrosis). They also did not have any contraindications for receiving an MRI such as metal devices in the body or claustrophobia. For full details of inclusion and exclusion criteria, see van Essen et al.³². Participants were excluded from the current analysis if they had missing data on cannabis use or for any covariates, resulting in loss of 32 participants. Thus, the final sample for this study comprised 1081 participants (Table 1).

Measures

Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II)

The cannabis use questions from the SSAGA-II³³ were used to assess lifetime exposure to cannabis. These items included *times used*, *age of first use*, and *lifetime CUD DX*. These responses were binned ordinally by the HCP research team prior to release to the public. *Times used* was binned into six categories: 1) never; 2) 1 to 5 times; 3) 6 to 10 times; 4) 11-100 times; 5) 101-999 times; and 6) 1000 or more times. *Age of first use* was binned into five categories 1) younger than 15; 2) 15-17; 3) 18-20; 4) over 20; and 5) never used. *Lifetime CUD DX* was coded as a binary variable.

THC Urine Screen

Participants completed a urine drug screen on the day of testing that assessed for recent exposure to tetrahydrocannabinol (THC), the psychoactive component of cannabis. From this a binary variable was created called *THC+ status* indicating whether participants were positive for the presence of THC in their urine. Prior research suggests that the amount of time an individual remains positive for THC is dependent on several factors including heaviness of cannabis use. Detection is possible for between one and three days after a single use and three to four weeks on average for heavy users, although it may be detectable up to three months later in some individuals³⁴.

Seven-day Retrospective Report of Tobacco and Alcohol Use

A seven-day retrospective report was given for tobacco and alcohol use. The candidate covariates current alcoholic drinks per week, heaviest alcoholic drinks per week, current number days of tobacco used per week, and lifetime cigarette smoking history were derived from this measure.

Procedures*MRI Data Acquisition and Processing*

High-resolution T1-weighted structural images were acquired on a 3T Siemens Skyra scanner (Siemens AG, Erlanger, Germany) with a 32-channel head coil at a resolution of 0.7 mm³ isotropic (FOV = 224 x 240, matrix = 320 x 320, 256 sagittal slices; TR = 2400 ms and TE = 2.14 ms). The quality checking procedure completed to ensure all scans were of high quality is documented in Marcus et al.³⁵. Data were reconstructed and preprocessed by the HCP team using the Freesurfer pipeline^{21,36} in FreeSurfer Image Analysis Suite version 5.3 (<http://surfer.nmr.mgh.harvard.edu>). In this process, Freesurfer models boundaries between the cortical white matter, gray matter, and pial surfaces in order to create a two-dimensional representation of the surface of the gray matter ribbon of the cortex. See HCP publications for more details of acquisition, reconstruction, and preprocessing^{37,38}.

Segmentation of Hippocampus and Amygdala

The standard FreeSurfer pipeline was utilized to process the MRI data and to derive intracranial volume (ICV) for use in this study^{21,36}. Hippocampal and amygdalar subfield segmentation was derived using the new automated algorithm available in FreeSurfer 6.0^{23,24}. The hippocampal and amygdalar segmentations are based on refined probabilistic atlases constructed by three independent neuroanatomists from both *in vivo* and *ex vivo* human brains

using both manual and automated methods. Using Bayesian inference, the atlas is used to automatically segment the hippocampus and amygdala into 9 bilateral nuclei (i.e., 18 total). These segmentation procedures were both validated on large publicly available datasets (ADNI and ABIDE) and both demonstrated greater accuracy in identifying individuals from special populations than prior versions of FreeSurfer.

Data Analysis

Prior to the primary analyses, to characterize the interrelation of cannabis use variables Pearson's correlations were conducted on the four cannabis use variables. Then hippocampal/amygdala subfield volume were also tested for association with each other using Pearson's correlations. In the primary analyses, each cannabis use variable was tested for association with volume in the hippocampus and amygdala using a two-step hierarchical approach. Both steps of this strategy used multiple linear regression with covariates entered into the model initially. Covariates included estimated total intracranial volume (eICV), age, gender, income, education, drinks per week, tobacco use days per week, and recent use of illicit drugs other than cannabis. These covariates were chosen for the known relationship with cannabis use and brain volume. Differences in covariates between cannabis users and non-users are shown in Supplemental Table 1. Additionally, twin status was controlled with two binary covariates: one for monozygotic twin status (yes/no) and one for dizygotic twin status (yes/no). This was done in accordance with other studies of the HCP dataset investigating cannabis use effects on brain volume^{39,40}. The first step of the primary analysis was to test the associations of each cannabis use variable with volume of the left and right whole hippocampus and volume of the left and right whole amygdala. In this step a threshold of $p < .005$ was used in accordance with recommendations from the literature⁴¹. In the second step, the hippocampus and amygdala were

divided into the subfields produced by Freesurfer 6.0. These included nine subfields per hemisphere for the hippocampus and nine per hemisphere for the amygdala, as well as the hippocampal head, hippocampal body, and hippocampal tail which are divisions that are orthogonal to the nine primary subfields derived. Then each of these subfields was examined for association with any of the cannabis use variables that were significantly associated with the whole amygdala or whole hippocampus for that hemisphere. In this step a false discovery rate threshold of $q < .05$ was used to account for family-wise error.

RESULTS

Preliminary Correlational Analyses

Cannabis use variables were all significantly correlated ($p < .001$; Supplemental Table 2). Additionally, bilaterally the whole hippocampus and whole amygdala were significantly associated with each of their own subfields ($p < .001$; Supplemental Figure 1).

Whole Hippocampus and Amygdala Associations with Cannabis Use

THC+ status and *time used* were both significantly negatively associated with both the left and the right whole hippocampus (Table 2). Additionally, *times used* was significantly negatively associated with left whole amygdala, but not the right whole amygdala. Neither *age of first use* nor *lifetime CUD DX* were associated with volume in either hemisphere of the amygdala or hippocampus.

Hippocampal Subfield Associations with Cannabis Use

THC+ status was significantly negatively associated with the left and right head of the hippocampus, though not the hippocampal body or tail (Table 3). Regarding hippocampal subfields, *THC+ status* was negatively associated with the left and right CA1, the left and right presubiculum and parasubiculum, the left dentate gyrus, and the right fimbria. *Times used* was

significantly associated with the left and right head of the hippocampus, though not the left hippocampal body or tail. Regarding hippocampal subfields, *times used* was associated with the left and right CA1, dentate gyrus, subiculum, and presubiculum, as well as the left HATA and right fimbria.

Amygdala Subfield Associations with Cannabis Use

Since only the left whole amygdala was associated with *times used*, only the left amygdala subfields were tested for association with *times used*. Regarding left amygdalar subfields, *time used* was associated with the left lateral nucleus, basal nucleus, paralamina nucleus, accessory basal nucleus, and corticoamygdaloid transition. Statistically significant associations were not present with any of the corticomedial subfields. Full statistics presented in Table 4.

DISCUSSION

The current results provide one of the highest resolution examinations of the effects of cannabis use on the volume of the hippocampus and amygdala to date. Results indicate negative associations of cannabis use with volume in the left and right hippocampus and in the left amygdala. Subfield analyses of the hippocampus indicate that these effects were being driven by negative associations between cannabis use and volume in the several hippocampal structures: the CA1, DG, parasubiculum, presubiculum, left subiculum and HATA, and right fimbria. Additionally, on the anterior/posterior axis of the hippocampus, cannabis use was associated with the hippocampal head, but not the body or tail. Subfield analyses of the left amygdala indicate these effects were driven by regions categorized in the basolateral complex the left lateral nucleus, basal nucleus, accessory basal nucleus, as well as the paralamina nucleus, anterior amygdaloid area, and corticoamygdaloid transition. Effects sizes of these associations were

small, with the largest effects found in the left hippocampal head and left CA1 ($R^2\Delta = .009$).

Regarding differences among the cannabis use variables, recent cannabis use (*THC+ status*) and total amount of cannabis used across the lifespan (*times used*) showed generally similar effects, which was not unexpected given the two cannabis measures were moderately correlated.

Both recent cannabis use and lifetime amount of use were associated with lower volume of the head of the hippocampus, but they were not associated with volume of the body or tail. The head of the hippocampus has specifically been associated with verbal working memory⁴². The hippocampal subfield most clearly associated with cannabis use was CA1, which was bilaterally associated with *THC+ status* and *times used*. CA1 receives inputs from the DG and CA3 along the perforant path. CA1 has been specifically linked to episodic memory¹⁴, as well as memory retrieval and consolidation^{15–19}, suggesting that those processes may be uniquely impacted by cannabis use. Additionally, the subfields that make up the subicular complex (subiculum, parasubiculum, presubiculum) were also associated with cannabis use. The subicular complex is the final stop in the perforant path receiving inputs primarily from CA1, but also from CA3 and DG. It is where outputs from the hippocampus are initiated, projecting outputs not only to the entorhinal cortex and perirhinal cortex, but also to the medial orbitofrontal cortex and anterior cingulate cortex^{43,44}. It has been linked repeatedly to spatial memory and it has been suggested that one of its roles is to amplify the signal from the rest of the hippocampus^{43,44}. It has also been suggested to be a part of the behavioral inhibition system and critical to the experience of anxiety⁴⁵. Thus, lower volumes in the subicular complex may compound any effects on emotion that result from reduced amygdalar volume in cannabis users; while this is necessarily conjecture, it would be consistent with research finding that cannabis users experience elevated rates of anxiety disorders relative to the general population⁴⁶. Additionally, the DG was

associated with times used bilaterally, but not with THC status, suggesting that this region may be more permanently affected by cannabis use. The DG is the primary site of inputs to the hippocampus, receiving inputs primarily from the entorhinal cortex⁴⁷. It is thought to have a distinct functional role from the rest of the hippocampus given its unique cytoarchitecture, possibly pertaining to the organization of inputs from the entorhinal cortex or rapid acquisition of spatial memories^{47,48}. However, more research is needed to understand why these specific subfields are related to cannabis use and the implications.

The volumes of the nuclei that make up the basolateral complex of the amygdala were inversely associated with the lifetime number of times cannabis had been used while the corticomedial nuclei were not associated with any cannabis use variable. The basolateral complex receives the majority of inputs into the amygdala, including the primary sensory pathways from the thalamus and secondary sensory pathways from polymodal association cortices, inputs from the hippocampus and entorhinal cortex, and inputs from the medial prefrontal cortex³⁰. The basolateral complex is also the primary source of output for higher order cognitive functions, with outputs going to the prefrontal cortex and polymodal association cortex that affect self-regulation and cognition. The basal amygdala also has outputs to the ventral striatum that are critical to the role of the amygdala in assigning positive affect to rewarding experiences. On the other hand, the corticomedial amygdala is the primary source of outputs for more visceral processes set off by the amygdala, with the central nucleus projecting to the hypothalamus, midbrain, and brainstem that impact arousal levels, parasympathetic nervous system activation, and freezing behavior in animals. Taken together, these results could suggest that cannabis use is more strongly associated with changes to the amygdala associated with sensory processing of emotional stimuli and the integration of emotional valence in higher-

order cognition, while it has less effect on the more visceral or emotional processes of the amygdala such as physiological arousal. However, this is by necessity highly speculative and more research is needed to understand why these specific subfields are related to cannabis use and what the implications of this are.

The differences in results between the cannabis use variables are notable. Changes to the hippocampus and amygdala were both related to *times used*, which is a measure of the total number of times used across the lifespan but includes no information about when these uses occurred. Conversely, no association was found between hippocampal or amygdalar volume and *age of first use*, which marks how early an individual began using cannabis but not about how much they have used. Thus, the current results suggest that the more important feature to the volume of these two structures is the amount of cannabis used rather than when it was used. Additionally, while the sheer quantity of use across the lifespan was inversely related to both hippocampal and amygdalar volume, developing a problematic pattern of use was not, as evidenced by the lack of associations of *lifetime CUD DX* with volume in either structure. This again suggests that the association is driven by the overall amount of cannabis used and not the behavioral pattern in which it was used. Finally, *THC+ status* was also associated with hippocampal volume suggesting that not only is the overall amount used, but the recency with which cannabis has been used is also an important factor. This is consistent with prior literature, which has found that differences in hippocampal volume found in cannabis users were not present in former cannabis users⁸. The current results provide some support for the hypothesis that hippocampal morphometry may revert to that of a non-user following prolonged abstinence from cannabis.

The present results differ from those of Gillespie et al.³¹ and Chye et al.²⁰, in which results indicated an absence of association between non-clinical levels of cannabis use and hippocampal or amygdalar morphometry. One likely reason for this is the improved resolution of the measurement of cannabis use in the current study, which measured cannabis use using both self-reported and biological measures, assessed for use both past and recent used, and distinguished between problematic and non-problematic cannabis use. The use of multiple cannabis measures presents one major improvement of this study compared to prior literature. Another improvement of the current study was the use of more advanced neuroimaging assessment using 3T MRI and Freesurfer's new automated hippocampal and amygdalar segmentation procedure^{23,24}, which was designed to address noted issues in existing automation segmentation procedures²². Additionally, this was the largest study to date to investigate these effects, which provided greater statistical power than previous studies. Each of these improvements may have allowed greater sensitivity to detect small effects. Indeed, the effect sizes of the associations of cannabis use and hippocampal/amygdalar morphometry were small, making prior lack of detection due to lack of power a possibility. Future large studies of heavy cannabis users may find larger effects, although this is conjecture.

Somewhat unsurprisingly, the whole hippocampus and amygdala results are generally consistent with prior reports using partial samples of the HCP dataset. Pagliaccio et al.³⁹ found associations of *times used* with the left amygdala volume and left hippocampal volume in a partial HCP sample. Likewise, Orr et al.⁴⁹ also found that the shape of the hippocampus and amygdala was associated with *times used* cannabis in a partial HCP sample. The current study extends the results of these studies by both verifying these aggregate results in the whole HCP

sample ($n = 1068$) and, more importantly, using a more advanced approach to segmentation to investigate the subfields of the hippocampus and amygdala.

One important issue relating to this analysis is the issue of directionality. Since this study uses a pseudo-experimental design, it cannot be definitively determined whether cannabis use modifies the volume of the hippocampus and amygdala, whether having smaller hippocampal and amygdalar volume is associated with a predisposition to use cannabis, or whether a third variable is associated with both cannabis use and volume of the hippocampus and amygdala. There is reason to suspect that hippocampal effects may be caused by cannabis use, rather than representing a predisposing factor to cannabis use. Associations of urinary *THC+ status* with hippocampal volume could suggest that cannabis use is causing changes to the volume of the hippocampus and that this change is reversible following abstinence. This is consistent with prior work that found that former cannabis users did not show differences in hippocampal volume⁸. If the association of cannabis use and hippocampal volume dissipates after cessation that would suggest it could not be a predisposing factor. On the other hand, *times used*, a lifetime frequency measure, does not speak to the directionality of the effect as the cannabis use; the cannabis use it describes could have occurred at any point in the lifetime and therefore may have preceded or followed the divergence in volume of these structures from non-cannabis users. This may suggest that the association of volume of the left amygdala with *times used* is not representative of a state effect, but rather a predisposition toward cannabis use in those with smaller left amygdala volumes. This would be consistent with a prior study conducted in a subsample of HCP participants that found evidence that the associations of cannabis use and the amygdala volume were being driven primarily driven by shared genetic factors³⁹.

This study has several important implications. First, the study's broader set of findings provide further evidence for a long held hypothesis that cannabis use is associated with smaller volumes in the hippocampus, the key structure in the formation and storage of memories, and the amygdala, which is the key structure in the processing and experience of emotion. This study provides one of the largest and most thorough test of this hypothesis to date, making it a meaningful contribution to the literature for this reason alone. Second, the results of subfield analyses provide clues for future research regarding the specific mechanisms by which cannabis use affects cognitive function. There is strong evidence that cannabis is associated with neuropsychological deficits^{40,50} and understanding the specific nuclei in the hippocampus and amygdala that differ in cannabis users provides a theoretical framework from which to base future research on the neural mechanisms by which cannabis affects these cognitive functions. For example, the consistent associations of the CA1 region of the hippocampus with cannabis use could suggest memory consolidation and retrieval as specific processes impacted by cannabis use for further investigation. Likewise, the association of the basolateral complex of the amygdala with cannabis use suggests that understanding the role of cannabis use on the integration of emotion with higher order cognitive processes may be a fruitful avenue for further exploration. Third, the differential results on cannabis use variables provide evidence about the nature of the effects cannabis may have on the hippocampus and amygdala. Findings indicating that recent use and total amount used lifetime are the most strongly associated features of cannabis use with hippocampal and amygdalar volume, as well as the lack of relationship between age of cannabis initiation and history of cannabis use disorder are telling regarding the putative relationship of cannabis and the brain. These results suggest that cannabis may have both acute and chronic effects on the volume of the hippocampus and amygdala, with permanent

effects being driven by overall amount of cannabis consumed across the lifespan, rather than, for example, being a result of early adolescent use.

One notable consideration of the current study is that of effect sizes. All associations demonstrated between cannabis use and volume of the hippocampus and amygdala were small. Despite findings that were significant substantially beyond appropriate multiple comparison correction, no cannabis use variable accounted for even 1% of the variance in the hippocampus or amygdala or in any subfield of either. However, even small effect sizes may have meaningful effects in some contexts, such as those already struggling academically or professionally due to limitations in memory who may be further impacted by these changes to the hippocampus. In addition, the effects are likely larger for individuals with very heavy cannabis use.

A limitation of this study was its inability to assess the specific behaviors associated with volume of the hippocampal and amygdalar subfields. Doing this would have required nuanced tests of memory and emotion which was not available given that the data used was drawn from an existing dataset. Future work exploring the differences in functional importance for these subfields is called for. Additionally, cannabis use is associated with numerous individual differences, which may serve as confounds in quasi-experimental research such as the current study. Fortunately, the current sample was well-powered enough to allow for extensive use of covariates, though it would be ideal to conduct this research in perfectly matched sample of cannabis users and non-users. Despite its limitations, the study had substantial strengths. This study does represent the largest investigation to date of the association between cannabis use and the subfields of hippocampus and amygdala. It used multiple cannabis use variables to determine which factors in cannabis use were most linked to differences in these regions. Additionally, this is one of the first studies to investigate the effects of cannabis use on hippocampal and

amygdalar subfields drawn using the new Freesurfer 6.0 segmentation procedure^{23,24} which improves up known issues in prior versions of Freesurfer in segmenting these structures²².

In summary, the current study demonstrates small but significant inverse associations between lifetime frequency of cannabis use and recent cannabis use with the volume of the hippocampus, specifically, in the head of the hippocampus, CA1, subicular complex, and DG. Additionally, it demonstrates similarly small but significant associations of lifetime cannabis use frequency and volume in the left amygdala, specifically in the subfields that are a part of the basolateral amygdala.

Table 1. Sample demographic characteristics ($N = 1081$). UDS=urine drug screen, CUD=Cannabis Use Disorder.

	Full Sample
Sex (%Female)	54.5%
Years of Age (mean)	28.8(3.7)
Race (% Non-White)	25.5%
Ethnicity (% Hispanic)	8.6%
Income (median/year)	\$50,000- \$74,999
Years of Education (mean)	14.9(1.8)
Drinks per Week (mean)	4.96(7.1)
Days Tobacco per Week (mean)	1.02(2.3)
Positive UDS for Illicit Drugs	0.6%
THC+ status (% positive)	11.0%
Times Used Cannabis (median)	1-10
Age 1 st Use (% adolescence)	26.4%
Lifetime CUD Diagnosis (% yes)	9.3%

Table 2. Associations of individual cannabis variables with whole hippocampus and amygdala volumes, covarying for estimated total intracranial volume, age, gender, income, education, twin status, drinks per week, tobacco use days per week, and recent use of illicit drugs other than cannabis. Bold indicates significant associations at $p < .005$.

Cannabis Index	Structure	<i>B</i>	<i>t</i>	<i>p</i>	<i>R</i> ² <i>Δ</i>
THC+ Status	Left Hippocampus	-0.07	-2.95	0.003	0.004
	Right Hippocampus	-0.07	-2.97	0.003	0.004
	Left Amygdala	-0.03	-1.40	0.16	0.001
	Right Amygdala	-0.03	-1.46	0.15	0.001
Times Used	Left Hippocampus	-0.09	-3.54	0.0004	0.006
	Right Hippocampus	-0.06	-2.64	0.009	0.003
	Left Amygdala	-0.07	-3.03	0.002	0.004
	Right Amygdala	-0.04	-1.76	0.08	0.001
CUD DX	Left Hippocampus	-0.02	-0.74	0.46	0.000
	Right Hippocampus	-0.01	-0.62	0.53	0.000
	Left Amygdala	-0.05	-2.14	0.03	0.002
	Right Amygdala	-0.02	-0.98	0.33	0.000
Age of First Use	Left Hippocampus	-0.05	-2.34	0.02	0.003
	Right Hippocampus	-0.04	-1.63	0.104	0.001
	Left Amygdala	-0.04	-1.97	0.049	0.002
	Right Amygdala	-0.02	-0.76	0.44	0.000

Table 3. Associations of *THC+ Status* and *times used* with hippocampal subfields, covarying for estimated total intracranial volume, age, gender, income, education, twin status, drinks per week, tobacco use days per week, and recent use of illicit drugs other than cannabis. Bold indicates significant at FDR $q < .05$.

Region (DV)	<i>THC+ Status</i>				<i>Times Used</i>			
	<i>B</i>	<i>t</i>	<i>p</i>	$R^2\Delta$	<i>B</i>	<i>t</i>	<i>p</i>	$R^2\Delta$
left hippocampal tail	-0.05	-1.53	0.13	0.002	-0.02	-0.63	0.53	0.000
left hippocampal body	-0.05	-1.88	0.06	0.002	-0.06	-2.25	0.02	0.003
left hippocampal head	-0.08	-3.18	0.002	0.005	-0.08	-3.08	0.002	0.005
right hippocampal tail	-0.04	-1.24	0.21	0.001	-0.01	-0.17	0.87	0.000
right hippocampal body	-0.03	-1.38	0.17	0.001	-0.05	-1.94	0.052	0.002
right hippocampal head	-0.08	-3.50	0.0005	0.006	-0.08	-3.08	0.002	0.005
left parasubiculum	-0.07	-2.36	0.02	0.004	-0.06	-1.96	0.05	0.003
left presubiculum	-0.07	-2.65	0.008	0.004	-0.10	-3.72	0.0002	0.008
left subiculum	-0.05	-1.92	0.055	0.002	-0.07	-2.72	0.007	0.004
left CA1	-0.08	-3.21	0.001	0.005	-0.11	-4.14	0.00004	0.009
left CA2/CA3	-0.02	-0.58	0.56	0.000	-0.05	-1.74	0.08	0.002
left DG	-0.06	-2.48	0.013	0.003	-0.08	-3.21	0.0014	0.005
left fimbria	-0.04	-1.31	0.19	0.001	-0.02	-0.66	0.51	0.000
left HATA	-0.05	-1.64	0.10	0.002	-0.07	-2.33	0.02	0.004
left hippocampal-fissure	-0.01	-0.42	0.67	0.000	-0.03	-1.04	0.30	0.001
right parasubiculum	-0.09	-3.11	0.002	0.007	-0.10	-3.07	0.002	0.007
right presubiculum	-0.09	-3.32	0.0009	0.006	-0.07	-2.41	0.02	0.003
right subiculum	-0.05	-1.79	0.07	0.002	-0.05	-1.88	0.06	0.002
right CA1	-0.08	-3.17	0.002	0.005	-0.07	-2.68	0.007	0.004
right CA2/CA3	-0.01	-0.38	0.70	0.000	-0.03	-1.13	0.26	0.001
right DG	-0.05	-1.89	0.06	0.002	-0.07	-2.66	0.008	0.004
right fimbria	-0.07	-2.52	0.012	0.004	-0.06	-2.09	0.037	0.003
right HATA	-0.06	-2.18	0.03	0.003	-0.03	-1.01	0.31	0.001
right hippocampal-fissure	0.01	0.45	0.65	0.000	-0.02	-0.77	0.44	0.000

Table 4. Associations of gray matter volume of amygdala segments on *times used*, covarying for estimated total intracranial volume, age, gender, income, education, twin status, drinks per week, tobacco use days per week, and recent use of illicit drugs other than cannabis. Bold indicates significant beyond false discovery rate $q < .05$.

	Region (DV)	<i>B</i>	<i>t</i>	<i>p</i>	<i>R</i> ² Δ
Basolateral Complex	left lateral nucleus	-0.07	-2.83	0.005	0.004
	left basal nucleus	-0.08	-3.12	0.002	0.005
	left accessory basal nucleus	-0.07	-2.64	0.008	0.004
	left paralaminar nucleus	-0.07	-2.56	0.011	0.003
	left corticoamygdaloid transition	-0.07	-2.57	0.010	0.004
Corticomedial Nuclei	left anterior amygdaloid area	-0.06	-1.93	0.054	0.003
	left central nucleus	-0.05	-1.64	0.10	0.002
	left cortical nucleus	-0.01	-0.26	0.80	0.000
	left medial nucleus	0.02	0.51	0.61	0.0002

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CONFLICTS OF INTEREST

MMO has no potential financial conflicts of interest to declare. LHS receives research funding from the National Institutes of Health. JM receives research funding from the National Institutes of Health, the Canadian Institutes of Health Research, and Correctional Service of Canada, and is a principal in BEAM Diagnostics, Inc.

AUTHOR CONTRIBUTIONS

Study concept was devised by MMO with assistance from JM and LHS. Statistical analysis and MRI data processing was conducted by MMO. Manuscript was primarily written by MMO with feedback provided by JM and LHS.

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