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Fatty Acid Amide Hydrolase is Lower in Young Cannabis Users

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Abstract

We have recently shown that levels of fatty acid amide hydrolase (FAAH), the enzyme that metabolises the endocannabinoid anandamide, are lower in the brains of adult cannabis users (34±11 years of age), tested during early abstinence. Here, we examine replication of the lower FAAH levels in a separate, younger cohort (23±5 years of age). Eighteen healthy volunteers (HV) and fourteen cannabis users (CU) underwent a positron emission tomography scan using the FAAH radioligand [^{11}C]CURB. Regional [^{11}C]CURB binding was calculated using an irreversible two-tissue compartment model with a metabolite-corrected arterial plasma input-function. The FAAH C385A genetic polymorphism (rs324420) was included as a covariate. All CU underwent a urine screen to confirm recent cannabis use and had serum cannabinoids measured. One CU screened negative for cannabinoids via serum and was removed from analysis. All HV reported less than five lifetime cannabis exposures more than a month prior to study initiation. There was a significant effect of group ($F(1, 26)=4.31$; $p=0.048$) when two A/A (rs324420) HV were removed from analysis to match the genotype of the CU group (n=16 HV, n=13 CU). Overall, [^{11}C]CURB λk_3 was 12% lower in CU compared to HV. Exploratory correlations showed that lower brain [^{11}C]CURB binding was related to greater use of cannabis throughout the past year. We confirmed our previous report and extended these findings by detecting lower [^{11}C]CURB binding in a younger cohort with less cumulative cannabis exposure.

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Author Contributions

RM designed the study. MJ, JW, TD, and RT conducted experiments. MJ and RM analyzed the data. MJ and RM wrote the manuscript, with input from IB, RT and JW. All authors critically reviewed the content and approved the final version for publication.

Disclosures

RFT has consulted for Quinn Emmanual and Apotex, and is a member of numerous scientific advisory boards, on unrelated topics. All other authors report no biomedical financial interests or potential conflicts of interest.

Keywords

addiction; anandamide; cannabis; [^{11}C]CURB; fatty acid amide hydrolase; positron emission tomography

Introduction

Cannabis is the most widely used illicit drug worldwide.[1] Its use is especially high among youth, with rates of use being 19% and 33% among youth aged 15–19 and 20–24, respectively, as compared to 13% in adults above 25 years of age, in Canada.[2] Meanwhile, the perceived risk of harm from cannabis use, by youth, has declined in recent years.[3] Roughly 10% of individuals who try cannabis become dependent.[4] However, approximately 15% of users become dependent if cannabis use is initiated during adolescence.[4] There are also reported associations between cannabis use and adverse effects including addiction, altered brain development, cognitive impairment, poor educational outcomes, and diminished life satisfaction.[4] Most of these findings appear to be more consistent when cannabis use is initiated in early adolescence.[4] Conversely, cannabis has been used for medicinal purposes in numerous ailments.[5]

The main psychoactive component of cannabis, (−)-trans-Δ9-tetrahydrocannabinol (THC), is a partial agonist for cannabinoid receptors 1 and 2 (CB1R and CB2R, respectively).[6,7] These receptors, along with their endogenous ligands and associated metabolizing enzymes, comprise the canonical endocannabinoid (eCB) system (although an extended eCB system has also been described).[8] Fatty acid amide hydrolase (FAAH), the catabolic enzyme responsible for controlling levels of fatty acid amides including anandamide, sets the tone of eCB signalling[9,10] and has garnered considerable attention as a potential therapeutic agent. Recently, D'Souza et al. (2019) showed treatment with FAAH inhibitor PF-04457845 to reduce cannabis withdrawal symptoms in men with cannabis dependence, highlighting FAAH's role in cannabis use disorders.[11]

Most,[12–15] but not all,[16] preclinical and post-mortem studies detect a decrease in CB1R availability with cannabinoid usage. In humans, positron emission tomography (PET) studies have consistently detected a downregulation of CB1R in cannabis users (CU);[17–19] these findings include a 20% reduction in the neocortex and limbic cortex,[17] a 15% reduction across brain regions,[18] and a global 12% decrease in CB1R availability.[19] However, this effect may be transient and reversible, normalizing after 2–4 days of abstinence.[18]

Data derived from research on endocannabinoids and their metabolizing enzymes are inconsistent. One study, which divided its cannabis users into "frequent" and "infrequent" users (those who used more and less than 10 times per month, respectively) found lower levels of anandamide in the frequent compared to infrequent users, but neither group differed from controls.[20] An additional study detected no effect of cannabis use on cerebrospinal fluid anandamide levels.[21] Using the novel PET radioligand [^{11}C]CURB, our centre reported a reduction of FAAH in the brains of adults (34±11 years of age) with cannabis use disorder (CUD) who had been using cannabis for 18±11 years.[22] Here, we aimed to replicate this

finding in a younger cohort with daily cannabis use, irrespective of CUD status. We measured FAAH in CU relative to healthy controls (HV) using [^{11}C]CURB and a high-resolution research tomograph (HRRT). We hypothesized that [^{11}C]CURB binding would be lower in the CU compared to HV across brain regions when tested in early abstinence as in our previous study. We also explored the relationship between [^{11}C]CURB binding and the extent of cannabis use and peripheral levels of cannabis metabolites.

Materials and Methods

Study Participants

Eighteen HV and 14 CU completed all study procedures. All participants were recruited using local advertisements in the Greater Toronto Area. Participants did not meet criteria for any DSM-IV Axis I disorder, as determined by the Structured Clinical Interview for DSM-IV (SCID),[23] with the exception of nicotine abuse/dependence, and cannabis abuse/dependence in CU. All participants were excluded for any of the following: first degree relatives with a psychotic disorder, significant current or past medical conditions, neurological illnesses or head trauma, use of medications that might affect the central nervous system, the presence of metal implants precluding an MRI scan, and/or pregnancy or breastfeeding. The Fagerstrom Test for Nicotine Dependence was used to assess nicotine dependence.[24]

CU were invited to participate if they smoked cannabis at least four days per week, had been using regularly for at least one year, and screened positive for cannabis use at baseline via urine toxicology. Hence, regular cannabis use, but not CUD, was required for inclusion criteria. However, DSM-5[25] was used to retrospectively determine probable CUD by using responses given on the SCID and the following questionnaires: Severity of Dependence Scale,[26] Drug Abuse Screening Test,[27] Marijuana Craving Questionnaire,[28] and Cannabis Withdrawal Scale.[29] The aforementioned scales were completed on scan day. All CU were asked to abstain from cannabis overnight (~12 hours) prior to the scheduled PET scan, as previously done by our group. At baseline, urine toxicology was used to rule out illicit drugs other than cannabis in CU. In addition, we collected blood samples for cannabinoid/metabolite analysis on the day of the PET scan (pre-scan). HV were required to have zero past exposure to street drugs besides cannabis, and no more than five lifetime cannabis uses, predating study initiation by at least one month.

This study was approved by the Research Ethics Board at the Centre for Addiction and Mental Health. All subjects provided written informed consent after being informed of all study procedures.

PET and MRI Data Acquisition and Analysis

[^{11}C]CURB PET data were acquired according to the validated method reported elsewhere.[30] [^{11}C]CURB was synthesized as previously described.[31] Briefly, participants underwent a transmission scan followed by an intravenous bolus injection of [^{11}C]CURB (9.81±0.72 mCi in HV, 9.50±0.86 mCi in CU) and 60 minute PET scan using a 3D HRRT brain tomograph (CPS/Siemens, Knoxville, TN, USA).[30] A 2D filtered-back projection algorithm, with a

HANN filter at Nyquist cutoff frequency, was applied to the 2D sinograms to reconstruct the images. Arterial blood samples were collected automatically using an automated blood sampling system (ABSS; Model PBS-101, Veenstra Instruments, The Netherlands) for the first 22.25 minutes post-injection at a rate of 2.5 mL/min, and samples were collected manually at 3, 7, 12, 20, 30, 45, and 60 minutes post-injection, to measure radioactivity in blood and determine the relative proportion of radiolabeled metabolites. A metabolite-corrected input function was generated as previously described.[30] Blood-to-plasma radioactivity ratios were interpolated using a biexponential function, and parent plasma fraction by a Hill function. To permit delineation of regions of interest, a standard proton density (PD) weighted brain magnetic resonance image (MRI) was acquired for each participant, using a Discovery MR750 3T MRI (General Electric, Milwaukee, WI, USA).

Time-activity curves for each region of interest (ROI) were extracted using an in-house imaging pipeline.[32] [^{11}C]CURB binding was quantified using the composite parameter λk_3 ($\lambda = K_1/k_2$), as derived from an irreversible two-tissue compartment model, which is the validated method for quantifying [^{11}C]CURB binding *in vivo*.[33]

rs324420 FAAH genotyping

The FAAH gene is polymorphic (rs324420, C385A), resulting in lower FAAH protein levels and associated [^{11}C]CURB binding [34] in carriers of one of more copy of the A allele. Thus, all participants were genotyped using a commercially available (Life Technologies, Burlington, Ontario, Canada) taqman assay as performed previously at our centre.[34]

Analysis of cannabinoid metabolites in serum

The cannabinoids multiplex assay (THC, THCOH, THCCOOH and CBD) was performed at the CAMH Clinical Laboratory by GC-MS (gas chromatography coupled with mass spectrometry) as described in the Varian (Agilent Technologies) application note 00315 with slight analytical modifications and CBD addition to the assay.[35] Results' validation was based on 3-level quality control (ACQ Science).

Statistical Analysis

Group differences in demographic measures were determined using independent sample t-tests for continuous variable and Fisher's exact tests for categorical variables. Group difference in [^{11}C]CURB λk_3 was analyzed using a linear mixed models analysis, with group as a between subjects factor, [^{11}C]CURB λk_3 as the dependent variable, ROI as a factor, and FAAH genotype as a covariate. The model tested for main effects of group, region, and genotype, and a group x ROI interaction. ROIs (12) included in the model were: cortical regions including prefrontal cortex (PFC), anterior cingulate cortex (ACC), temporal cortex, occipital cortex, parietal cortex, and insula, and subcortical regions including dorsal striatum, ventral striatum, hippocampus, amygdala, thalamus, and cerebellum. Associations between [^{11}C]CURB λk_3 and cannabis use measures were tested using bivariate Pearson correlations. Further, we ran partial correlations to explore these associations including covariates (hours of cannabis abstinence, THC, and THCCOOH levels) to control for the potential confounding effects of recent use and cannabis use history. [^{11}C]CURB λk_3 values used in all correlations were adjusted for genotype by running a linear regression to generate

the residuals of λk_3 values of each ROI on FAAH genotype. A genotype-corrected whole brain [^{11}C]CURB λk_3 value was generated by calculating a weighted-average value representative of all 12 ROIs. All statistical analyses were performed using SPSS (version 24.0; IBM, Armonk, NY, USA), with $p<0.05$ considered to be significant. Bonferroni corrections were completed for correlations in a priori regions by dividing the alpha level (0.05) by the number of comparisons (four, corrected alpha=0.013).

Results

Participant demographics

Eighteen HV and 14 CU performed all study procedures. One CU screened negative for serum THC/metabolites on scan day and was thus removed from analyses (n=13 CU). There was no significant difference in the frequencies of C385A FAAH genetic polymorphism (rs324420) between groups (n=18 HV,13 CU, p=0.71). As previously demonstrated at our centre,[34] there was a significant effect of genotype on [^{11}C]CURB binding ($p<0.05$), whereby binding is lower in carriers of the C385A variant. Therefore, the 2 HV who were homozygotes for the A allele were removed from analysis to improve the match to the CU group, which did not consist of any individuals who were homozygous for the A allele.

Group demographics are reported in Table 1 (n=16 HV, n=13 CU). The groups did not differ with respect to age, sex, ethnicity, BMI, or total years of education (all $p>0.05$). In addition, there were no significant differences between groups for any of the PET radiotracer parameters, including injected radioactivity, mass injected, and specific activity at the time of synthesis. There was a higher number of daily nicotine users in the CU group than the HV group; only one met criteria for nicotine dependence by scoring at least a four on the Fagerstrom Test for Nicotine Dependence. There was no difference in number of cannabis uses between the nicotine users and non-users in the week (p=0.35) or year (p=0.82) prior to study.

All CU screened positive for cannabis use via urine toxicology at baseline. Cannabis use is detailed in Table 2. THC (range:1.3–35.5 ng/mL, mean:10.6± 9.8 ng/mL, n=13) and its metabolite 11-nor-9-carboxy-THC (THCCOOH; range: 10–308 ng/mL, mean: 101.9± 92.3 ng/mL, n=13) was detected in the serum of 13 CU on scan day. For the 13 CU included in analysis, the average age of first consistent cannabis use (at least weekly) was 17.9±5.5 (13–33 years of age), and they had been using cannabis regularly for an average of 4.8 ±2.7 years (1–9 years). At the time of study, the 13 CU reported using cannabis on average 6.9±0.3 days (6–7) out of the past week and 28.2±3.4 days (24–30) out of the past 30 days. We retrospectively determined that 6 of the 13 CU met DSM-5 criteria for CUD. All CU reported fewer than 15 lifetime exposures to any other illicit drugs, with the most recent report being one instance of use, which occurred one month prior to the PET scan. Only two HV reported one lifetime use of cannabis (more than 30 days prior to the time of study), and all HV reported zero lifetime exposures to other illicit drugs. The average time of self-reported cannabis abstinence prior to the PET scan was 13.0±2.5 hours (6.8–14.8 hours). There were no significant correlations between hours of abstinence and genotype-adjusted [^{11}C]CURB λk_3 any in ROI (all $p>0.05$).

[^{11}C]CURB binding in cannabis users during early abstinence

We detected a significant effect of group (F(1, 26)=4.31; p=0.048), genotype (F(1, 26)=6.54; p=0.021), and ROI (F(11,297)=41.84; p<0.00), and no significant group x ROI interaction (F(11,297)=1.057; p=0.40), with the removal of the two A/A HV. Overall, [^{11}C]CURB λk_3 was 12% lower in CU (n=13) compared to HV (n=16). In individual ROIs, the difference in [^{11}C]CURB λk_3 between CU and HV ranged from 8.5% in the insula to 16.0% in the hippocampus (Figure 1). There was no effect of sex (F(1,25)=0.05; p=0.83), tobacco use (F(1,25)=1.218; p=0.28), or past use of street drugs excluding cannabis (F(1,25)=.182; p=0.67) on [^{11}C]CURB λk_3. There was no difference in [^{11}C]CURB λk_3 between the CU who we determined to have CUD (n=6) and those without, in this small sample (n=7; F(1,10)=0.10; p=0.76). When the analysis was performed including the HV with the A/A genotype (n=18 HV, n=13 CU), there was a trend towards lower [^{11}C]CURB binding in the CU compared to HV (F(1,28)=3.50; p=0.072), with an effect of ROI (F(11,319)=43.14; p<0.00) and genotype (F(1,28)=20.69; p<.00), and nonsignificant group x ROI interaction (F(11,319)=0.84; p=0.60).

As expected, the sample was heterogeneous with respect to cannabis use. One participant displayed serum THC levels that indicate recent use (>25ng/mL), although they did not report using cannabis closer to the scan. Another CU reported using cannabis ~6.5 hours prior to the scan, although they were instructed to abstain for 12 hours prior to the scan (serum THC levels, 17.4 ng/mL, were within the range of the rest of the group, 1.3–35.5 ng/mL). A sensitivity analysis found that with the removal of this individual, (n=12 CU, n=16 HV), [^{11}C]CURB λk_3 was 8.5% lower in CU compared to HV, but this effect did not reach statistical significance (F(1,25)=2.892; p=0.10). Similarly, with removal of the individual with high levels of serum THC (>25 ng/mL), binding was 14.7% lower in CU (n=12) compared to HV (n=16), but the effect was not statistically significant (F(1,25)=3.922; p=0.059), likely due to small sample size. There was no main effect of hours since cannabis use (F(1,11)=2.557; p=0.14), or serum levels of THC (F(1,11)=0.803; p=0.39) or THCCOOH (F(1,11)=0.001; p=0.98) on [^{11}C]CURB λk_3.

Exploratory association between [^{11}C]CURB λk_3 and cannabis use

Correlations were run between cannabis use measures and [^{11}C]CURB λk_3 in a priori regions of interest, including the amygdala, hippocampus, and ACC, as these regions were previously found to correlate with THC metabolites, and the ventral striatum because of its relevance for drug abuse/dependence.[36] After performing a Bonferonni correction for four comparisons (corrected α=0.013), only the correlation between estimated number of days smoked in the past year, as determined using the Drug History Questionnaire, and [^{11}C]CURB λk_3 in the ventral striatum (r=−.672; p=0.012) remained significant (Figure 2; n=13). This correlation remained significant after correcting for the hours since last cannabis use (r=−0.730; p=0.007), the THC in serum (r=−.767; p=0.004), and THCCOOOH in serum (r=−.772; p=0.003). The correlation between number of days used in the past year and [^{11}C]CURB λk_3 in the amygdala was also significant when controlling for THC (r=−0.806; p=0.002) and THCCOOH (r=−.711; p=0.006) in serum. An exploratory correlation was also detected between number of days smoked in the past year and an ROI-weighted whole-brain average λk_3 value (r=−.628; p=0.022), which remained significant (p<0.05) when

controlling for THC/THCCOOH/time since last use. There were correlations between [^{11}C]CURB binding and THCCOOH/THC (ratio, as validated in Huestis et al., 1992)[37] across regions, including the amygdala (r=0.662; p=0.031), ventral striatum (r=0.599; p=0.040), ACC (r=0.597; p=0.040) and hippocampus (r=0.501; p=0.097). These exploratory correlations were not corrected for multiple comparisons. There were no significant correlations between [^{11}C]CURB binding and any measures from questionnaires relating to cannabis use (craving/withdrawal).

Discussion

FAAH in CU during early abstinence

Our current findings are in line with the prior study, which detected lower levels of [^{11}C]CURB binding (λk_3) in adults with CUD measured during early abstinence.[22] In the current study, we detected lower [^{11}C]CURB binding in CU compared to HV similarly tested in early abstinence. It is possible that recent cannabis use affects [^{11}C]CURB binding such that FAAH is lower with recent use, but not after longer abstinence. This should be confirmed in larger studies designed to assess the impact of abstinence on [^{11}C]CURB binding. Notably, the percent difference in [^{11}C]CURB binding between groups in the current study (8.5–16.0%, CU n=13) is lesser than previously reported (14–20%).[22]

The CU in the current and previous studies differed with respect to their ages and cumulative lifetime cannabis use. The subjects in the previous study[22] and the current study did not differ with respect to age of cannabis use initiation (17.9±5.5 and 16.2±4.2) or grams of cannabis used in the past week (10.8±6.6 and 10.4±6.4), past 90 days (85.7±7 and 76.4 ±15) or in the past year (300.0±70 and 305.6±60.1), for the current and previous studies, respectively. However, our cohort of CU was aged 23±5 years, which is younger than the previous cohort that had a mean age of 34±11 years (p=0.010). In addition, the CU in the present study had on average 4.8 ±2.7 years of regular cannabis use, less than the 17.5±10.8 years of use in the previous cohort (p=0.010). Also, unlike in the previous study, not all participants in the current study met DSM-IV/5 criteria for CUD. In the current study, there was no difference in [^{11}C]CURB binding between CU who were determined to meet DSM-5 criteria for CUD (n=6) versus those who did not (n=7), though this is likely due to the small sample size. Due to the similar cannabis use reported in the two samples at the time of study, it is unlikely that frequency of use accounts for the difference in magnitudes between groups. Therefore, the discrepancies in results between the current and previous studies may be attributed to the differences in cumulative use over a number of years, or CUD status. Furthermore, our sensitivity analysis highlights the importance of considering cannabis use patterns on [^{11}C]CURB binding. The different ages of the CU in the two studies might be another potential explanation for the discrepancy between the observed [^{11}C]CURB λk_3 group differences. Overall, our findings highlight the importance of considering the extent of cannabis use/CUD diagnosis when discussing the effects of cannabis use on brain FAAH, and future research should seek to disentangle the effects of age, CUD, and years of cannabis use, on FAAH.

To our knowledge, there is limited research on how cumulative cannabis use affects the eCB system in humans. Hirvonen et al. (2012) reported an inverse association between CB1R

availability and years of cannabis use, with a greater reduction apparent in those with more years of use.[17] However, subsequent studies of CB1R in CU failed to detect such an association.[18,19] In rodents, the downregulation of CB1R in response to cannabinoid exposure is dose-dependent.[12] It is possible that FAAH is sensitive to the amount of total cumulative cannabis use. In fact, Boileau et al. (2016) detected an inverse association between levels of THC metabolites and [^{11}C]CURB λk_3, suggesting that heavier recent cannabis use is associated with lower FAAH levels. Coincidentally, we reported a negative association between past-year cannabis use and ventral striatum [^{11}C]CURB λk_3 (discussed below).

Prior to the first study at our centre,[22] the existing literature regarding the relationship between FAAH and cannabis use was ambiguous. Studies that measured anandamide levels in the cerebrospinal fluid of CU detected no alteration compared to healthy volunteers.[20,21] Genotype studies reported that individuals with the FAAH A/A genotype, associated with lower FAAH expression/activity,[38,39] might be at the lowest risk of becoming cannabis dependent,[40] and the high expression/activity C/C genotype has been linked with other cannabis-related problems.[41–43] Whereas these studies suggest that higher levels of FAAH might predispose individuals to CU dependence and associated problems, our finding suggests that FAAH levels are lower in CU in early abstinence.

A number of interactions in the eCB system could account for the lower observed FAAH in early abstinence CU. It is possible that in response to the decrease in CB1R availability in CU,[17–19] FAAH is reduced to increase anandamide and subsequent CB1R signalling. A second possibility is that the lower FAAH in CU is a compensatory mechanism to account for a downregulation of anandamide in CU. Alternatively, given that THC has been shown to suppress immune responses in cell models,[44] Boileau et al. (2016) proposed that CU might exhibit lower FAAH alongside a decrease in microglia activity/density; however, findings from our group do not support this postulation.[45]

Although we failed to reproduce the correlations between serum levels of THC or THCCOOH and [^{11}C]CURB λk_3 as previously reported,[22] we were able to detect correlations between the THCCOOH/THC ratio and [^{11}C]CURB λk_3 across regions. A difference in methodology for the quantification of the THC/metabolites, in serum in the present study, and whole blood in the previous study,[22] could contribute to the discrepancies in metabolite levels and relationships to [^{11}C]CURB binding. Importantly, we did not obtain a second serum sample (post-scan), which could indicate whether CU were in early abstinence, which was established when the previous relationship was observed.[22]

Exploratory correlations with cannabis use measures

Here, we explored the relationship between cannabis use and [^{11}C]CURB binding. We observed negative correlations between estimated days smoked in the past year and [^{11}C]CURB λk_3 in the ventral striatum, and both the ventral striatum and amygdala when controlling for serum THC/THCCOOH levels. These correlations were corrected for four comparisons and may not survive additional corrections. Our results, which control for FAAH genotype, suggest that the observed lower levels of FAAH is potentially related to the extent of cannabis use in the preceding year, and not solely as a result of recent use, or of

predisposition. This relationship between cumulative cannabis use and FAAH is especially interesting when considering the importance of cannabis dose/extent of usage and its adverse effects. For example, meta-analysis revealed that the risk of psychotic outcomes from cannabis use is dependent on the extent of cumulative cannabis use.[46] Furthermore, frequency and amount of cannabis has been correlated to the degree of cognitive effects from cannabis, such as verbal learning and memory in adolescent CU.[47] Our finding, which associates heavier cannabis use with lower [^{11}C]CURB binding in the ventral striatum, is interesting when considering both the role of ventral striatum in reward/addiction,[35] and FAAH as a target for cannabis use disorder treatment.[11] All of the associations between days smoked in the past year and [^{11}C]CURB λk_3 are exploratory, given the small sample size and the number of correlations. Nevertheless, these findings highlight the importance of cumulative cannabis use on the eCB system.

Limitations

Strengths of this study include the use of an HRRT and a radiotracer with excellent reproducibility and reliability.[33] Although we cannot confirm that [^{11}C]CURB λk_3 is representative of FAAH enzyme activity, [^{11}C]CURB binds to FAAH at the catalytic site for anandamide hydrolysis, so binding of [^{11}C]CURB to FAAH is reflective of available FAAH catalytic sites, suggesting a role in enzyme activity.[30,48] Further, validation from our centre found [^{11}C]CURB binding (λk_3) to be independent of cerebral blood flow.[30] One limitation of this study is that CU were not specifically screened for CUD using DSM-5 (although we did retrospectively determine some participants to meet criteria for CUD using DSM-5 and additional questionnaires). Nonetheless, all CU reported high levels of cannabis use (using 6.9±0.3 days in the past week) at the time of study. As with most studies in CU, lifetime and recent cannabis metrics were based on self-report and do not distinguish between cannabis strains or potencies. In addition, we obtained only one serum sample on scan day (pre-scan), and thus we are not able to discern whether our study participants were in early abstinence (~12 hours) as verbally reported. Also, participants were not excluded for use of caffeine, nicotine, and/or alcohol, and a detailed history of alcohol use was not obtained for each participant. However, there was no main effect of tobacco use on [^{11}C]CURB λk_3, and only one participant was nicotine-dependent according to the Fagerstrom Test for Nicotine Dependence. In addition, no participants met DSM-IV/5 criteria for alcohol use disorder. The sample size was also small and not fully matched between groups, which prompted the exclusion of the two A/A subjects from the HV group, to better match the groups. Although an a priori power analysis was not conducted as all participants were recruited as part of a larger study, a power analysis based on the previously published study with effect size d=0.96[22] suggests that a sample size of 38 (19 participants per group) would be required to detect a group difference with an alpha level of 0.05 and 80% power. We therefore decided that a small sample size would be sufficient to conduct this study given it's replicative nature, while minimizing the number of volunteers who would be exposed to radiation. Finally, since the results were obtained during (presumed) early abstinence (13.0 ±2.5 hours), it is unknown if the reduction of FAAH remains over a longer period of abstinence.

Conclusion

We extended previous findings[22] by performing the current study in a cohort that was younger and had less cumulative cannabis use than previously reported. We report a small group difference, yet significantly lower [^{11}C]CURB binding in CU compared to HV in the current sample. We highlight the importance of extent of cannabis use when considering its potential effects on the eCB system. Our results suggest that the eCB system could be altered in younger individuals who have less cumulative lifetime use of cannabis. While small, we believe our findings are important, especially considering recent legalization of recreational cannabis use across the globe. Our sample captured individuals in their early-mid-twenties, an age where there is elevated risk for developing CUD[49] and psychotic disorder(s),[50] alongside widespread recreational cannabis use.

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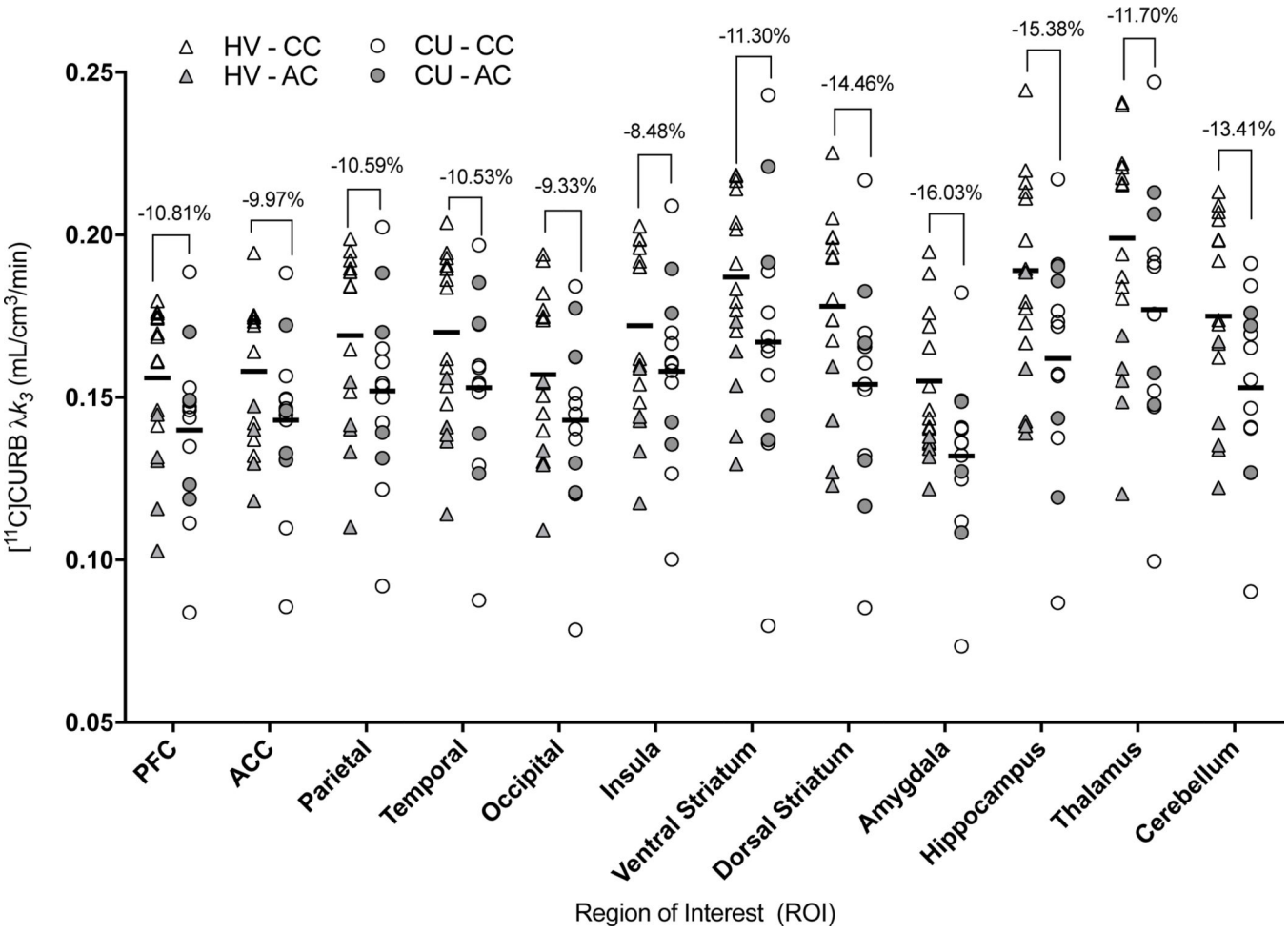

Figure 1.

Brain [^{11}C]CURB λk_3 values, an index of [^{11}C]CURB binding to fatty acid amide hydrolase (FAAH), for each participant in the HV (triangles) and CU (circles) groups. The C/C and A/C FAAH genotypes (C385A, rs324420) are indicated by white and grey symbols, respectively. The group means presented are the adjusted means to account for FAAH genotype. Percent differences are displayed for each ROI in [^{11}C]CURB λk_3 in CU (n=13) vs HV (n=16).

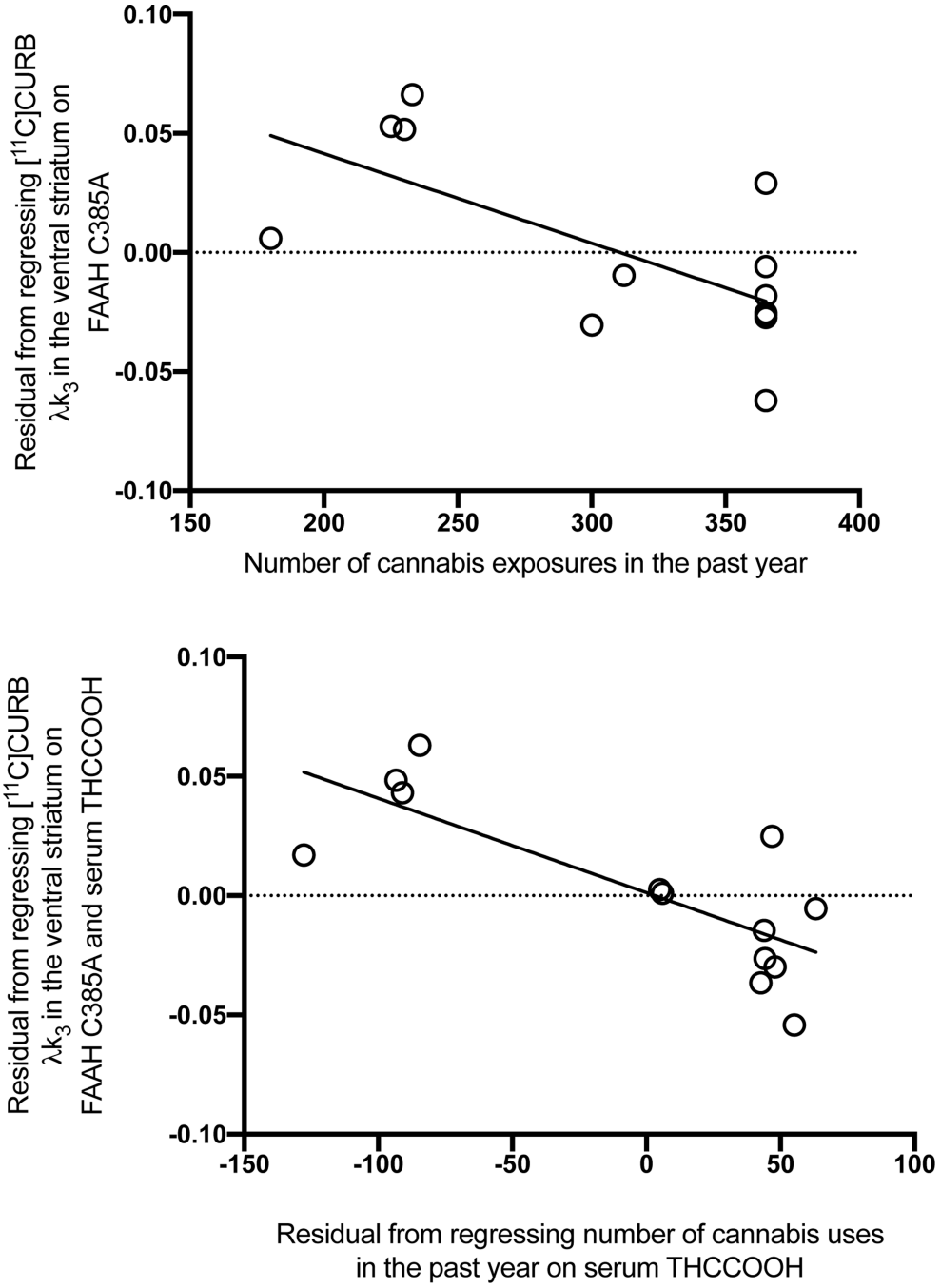

Figure 2.

Correlation between cannabis use in the past year and [^{11}C]CURB λk_3 in the ventral striatum and (n=13). All [^{11}C]CURB λk_3 values are all corrected for the FAAH C385A single nucleotide polymorphism. In the bottom plot, the values on the x and y-axis reflect a partial correlation, which was corrected or serum THCCOOH levels.

Table 1.

Participant Demographics

Measure		HV* Mean (S.D)	CU* Mean (S.D)	Result	Sig.
n		16	13	-	-
Age		21.77 (1.93)	22.88 (4.58)	−.87	.43
Sex	**Female**	8	4	-	.45
	Male	8	9		
FAAH Genotype (FAAH C385A polymorphism rs324420)	**CC**	11	9	-	1.00
	AC	5	4		
	AA	0	0		
Daily nicotine users		0	5	-	.01
BMI		24.10 (4.78)	24.23 (4.60)	−.40	.97
Years of education		14.78 (1.86)	13.50 (2.68)	1.33	.20
Ethnicity	**White**	7	7	-	.71
	Asian	5	2		
	Black	4	4		
Radioactivity injected (mCi)		9.86 (0.66)	9.50 (0.86)	1.76	.91
Molar activity at time of injection (mCi/μmol)		2086.60 (749.19)	1932.43 (706.68)	.74	.47
Mass injected (μg)		1.69 (0.72)	1.78 (0.85)	−.36	.73

*Demographics are shown for HV n=16, after the removal of the 2 HV with the A/A FAAH genotype, in order to better match groups. This cohort of HV was used for all calculations, unless otherwise stated. One CU was removed from analysis after screening negative for THC/metabolites in serum on scan day (n=13).

Table 2.

Cannabis Use Measures

Measure (n=13)	Mean (S.D)	Range
THC in serum (ng/mL)	10.6 (9.8)	1.3–35.5
THCCOOH in serum (ng/mL)	101.9 (92.3)	10–308
Age of first cannabis exposure	16.4 (4.3)	13–29
Age of first consistent cannabis use	17.9 (5.5)	13–33
Years of regular cannabis use	4.8 (2.7)	1–9
Estimated days of cannabis use (past year)	300.0 (70)	180–365
Estimated days of cannabis use (past month)	28.2 (3.4)	24–30
Cannabis smoked in the past week (grams)	10.8	4–25
Number of days of cannabis use in the past week	6.9 (0.3)	6–7
SDS (Severity of Dependence Scale)	3.1 (2.3)	0–7
DAST (Drug Abuse Screening Test)	6.5(4.2)	0–15
MCQ (Marijuana Craving Questionnaire)	42.2(9.6)	25–57
-Compulsivity	6.2 (2.4)	3–10
-Emotionality	8.8 (3.8)	3–15
-Expectancy	11.5	6–17
-Purposefulness	15.6 (5.4)	3–21
CU who were identified to have probable CUD (n)	6	

Drug use profiles of CU who screened positive for cannabinoids in serum on screen day (n=13). This sample was included in all analyses, unless otherwise stated.