ABSTRACT

BACKGROUND: One of the major mechanisms for terminating the actions of the endocannabinoid anandamide is hydrolysis by fatty acid amide hydrolase (FAAH), and inhibitors of the enzyme were suggested as potential treatment for human cannabis dependence. However, the status of brain FAAH in cannabis use disorder is unknown.

METHODS: Brain FAAH binding was measured with positron emission tomography and [11C]CURB in 22 healthy control subjects and ten chronic cannabis users during early abstinence. The FAAH genetic polymorphism (rs324420) and blood, blood, and hair levels of cannabinoids and metabolites were determined.

RESULTS: In cannabis users, FAAH binding was significantly lower by 14%–20% across the brain regions examined than in matched control subjects (overall Cohen’s d = 0.96). Lower binding was negatively correlated with cannabinoid concentrations in blood and urine and was associated with higher trait impulsiveness.

CONCLUSIONS: Lower FAAH binding levels in the brain may be a consequence of chronic and recent cannabis exposure and could contribute to cannabis withdrawal. This effect should be considered in the development of novel treatment strategies for cannabis use disorder that target FAAH and endocannabinoids. Further studies are needed to examine possible changes in FAAH binding during prolonged cannabis abstinence and whether lower FAAH binding predates drug use.

Keywords: Cannabis use disorder, [11C]CURB, Endocannabinoid, FAAH, Fatty acid amide hydrolase, Positron emission tomography

http://dx.doi.org/10.1016/j.biopsych.2016.04.012
preclinical data (31,32). These findings suggest that heavy chronic cannabis exposure might be associated with elevated brain FAAH activities (33,34) and that targeting FAAH inhibitor might be useful for cannabis withdrawal. Indeed, the FAAH inhibitor UR597 reduced rimonabant-precipitated withdrawal in THC-dependent mice (17). Large-scale genotyping studies showed that the functional variant of FAAH (rs324420, C385A) (35,36) associated with lower AEA concentrations (the C allele) is related to increased CUD risks (37). Other studies showed that the C allele is increased with increased self-reported positive feeling during a marijuana challenge (38), craving during abstinence (39), cue-induced reward circuit activation (40), and negative mood symptoms linked to frontolimbic white matter abnormalities (41). Of note, we recently showed that the C/C genotype has higher levels of FAAH binding in the brain with the use of our positron emission tomography (PET) tracer $^{[11C]}$CURB ([11C-carbonyl]-6-hydroxy-[1,10-biphenyl]-3-yl cyclohexylcarbamate; URB694) (42).

Currently, there are no studies investigating brain FAAH in living humans chronically exposed to cannabis. The recently developed PET tracer $^{[11C]}$CURB (43) is an effective PET radioligand, which binds selectively and irreversibly to FAAH (44). We recently reported that FAAH binding can be estimated reliably and reproducibly (44) with the use of a two-tissue compartment model with irreversible trapping that fitted regional time-activity curves with the composite variable $k_3$ ($\lambda = K_a/k_d$) as the preferred index of FAAH binding (45).

The aim of the present study was to use $^{[11C]}$CURB PET to investigate possible differences in FAAH binding in chronic cannabis users relative to healthy control subjects. Cannabis users were examined after acute overnight cessation of cannabis use, matching the first time point of the CB1 PET imaging study of Hirvonen et al. (20). We hypothesized that $^{[11C]}$CURB $k_3$ would be higher in the brain of cannabis users. Quantitative measurement of cannabinoids and metabolites is highly valuable for understanding and monitoring the presence and extent of cannabis use and withdrawal (46–51). For these reasons, blood and urine samples were collected during the scan for assays of cannabinoids concentrations.

METHODS AND MATERIALS

Subjects

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board. Subjects were recruited from the local community in Toronto, Ontario, Canada, using Internet advertisements to participate in a single $^{[11C]}$CURB PET scan. After provision of written informed consent, subjects completed a comprehensive screening session to rule out past or present significant medical conditions, neurologic illnesses, head trauma, Axis I psychiatric disorders, magnetic resonance and PET contraindications, use of medications that may affect the central nervous system, or positive screening for drugs of abuse except for cannabis in cannabis users. Scalp hair samples, if available, were taken for forensic drug analyses by liquid chromatography–mass spectrometry or gas chromatography–mass spectrometry at the United States Drug Testing Laboratories (Des Plaines, IL), including cannabinoids [11-nor-9-carboxy-THC (THCCOOH)], cocaine, amphetamines, and opiates.

Subjects were asked not to drink caffeinated beverages on the morning of the scan. Cannabis users were required not to use cannabis for 12 hours (overnight) before scanning (same requirement for nicotine smoking). PET intake assessments included 1) a breath alcohol concentration measurement to ensure abstinence from alcohol (0 required for scanning); 2) a urine toxicology to rule out medication and illicit drug use (other than cannabis in cannabis users); 3) urine pregnancy test (female subjects only); 4) expired carbon monoxide (<10 ppm to rule out recent tobacco or cannabis smoking); 5) craving and withdrawal questionnaires for cannabis users only [Severity of Dependence Scale, Obsessive Compulsive Smoking Scale, Marijuana Craving Questionnaire-Short Form (52), and Marijuana Withdrawal Checklist (53)] and the Time Line Follow Back to assess cannabis use over the previous 90 days (54); and 6) the Barratt Impulsiveness Scale (BIS) to measure trait impulsivity that has been related to eCS and greater cannabis-related problems in cannabis users (as a moderator of this relation) (55,56).

Analysis of Cannabinoids and Metabolites in Whole Blood and Urine

On the day of the PET scan for cannabis users, whole blood (in gray-topped Vacutainers [BD-Canada, Mississauga, ON, Canada] that contained potassium oxalate and sodium fluoride) and urine samples were collected twice (T1, on arrival; T2, before discharge), with the interval approximately 5 to 6 hours. The samples were transferred to polypropylene cryotubes, frozen on dry ice, stored at –80°C, and shipped within 3 months to Dr. Huestis’ laboratory at the National Institutes of Health for quantification of cannabinoids and metabolite concentrations. THC, 11-hydroxy-THC (11-OH-THC), THC-glucuronide (THC-gluc), THCCOOH, THCCOOH-glucuronide (THCCOOH-gluc), cannabidiol (CBD), and cannabiol (CBN) were quantified in blood by liquid chromatography–tandem mass spectrometry (46–51,57), with limits of quantification (LOQs) of 1 µg/L for THC, 11-OH-THC, THCCOOH, CBD, and CBN; 0.5 µg/L for THC-gluc; and 5 µg/L for THCCOOH-gluc. In urine, THC, 11-OH-THC, THCCOOH, CBD, and CBN were quantified by two-dimensional (2D) gas chromatography–mass spectrometry after alkaline hydrolysis, with LOQs of 2.5 µg/L for THC, 11-OH-THC, CBD, and CBN and 5 µg/L for THCCOOH.

Image Acquisition and Reconstruction

$^{[11C]}$CURB radiosynthesis was described previously (43). PET was performed with a three-dimensional brain high-resolution research tomograph (CPS/Siemens, Knoxville, TN) [see (45) for details of image acquisition]. In brief, after the subject lay down on the scanning table with head held in place with a thermoplastic mask to reduce movement, a short transmission scan was acquired, followed by injection of 370 ± 40 MBq (10 ± 1 mCi) of $^{[11C]}$CURB (58). Brain radioactivity was measured during sequential frames of increasing duration. Scanning time was 60 minutes. Images were reconstructed from the 2D sinograms with a 2D filtered-back projection algorithm, with a HANN filter at Nyquist cutoff frequency. After injection, arterial samples were manually collected from a radial artery at 3, 7, 12, 20, 30, 45, and 60 minutes and
automatically for the first 22.25 minutes (automatic blood sampling system, Model PBS-101; Veenstra Instruments, Joure, The Netherlands). A metabolite-corrected plasma curve was generated and used as the input function for the kinetic analysis [details in Rusjan et al. (45)]. Blood-to-plasma radioactivity ratios were interpolated by a biexponential function and parent plasma fraction by a Hill function. Subjects underwent standard proton density–weighted brain magnetic resonance imaging on a Discovery MR750 3T magnetic resonance imaging scanner (General Electric, Milwaukee, WI) for the purpose of region of interest (ROI) delineation and also an arterial spin labeling (ASL) sequence for cerebral blood flow measurement (45).

**ROI Kinetic and Statistical Analysis**

Time-activity curves acquired over 60 minutes [according to Rusjan et al. (45)] in each ROI were extracted with ROMI [details in Rusjan et al. (59)] and analyzed by a two-tissue compartment model with irreversible binding to the second compartment (45). The variable of interest to quantify FAAH binding is the composite variable $k_{3}$. Differences in [$^{11}$C]-CURB $k_{3}$ in each ROI between the two groups was investigated with analyses of variance (ANOVA) with ROI as a within-subject factor and group as a between-subjects factor (SPSS 21.0, SPSS Inc., Chicago, IL). When appropriate, least significant difference $t$ tests, Bonferroni corrected, were applied to determine the significance of regional differences in [$^{11}$C]CURB binding between groups. Data for healthy controls ($n = 22$) were previously published as part of the genetic association study of FAAH rs324420 on [$^{11}$C]CURB binding (42).

**FAAH Genotyping**

Given the importance of genotype on FAAH (and CURB binding) (42), all subjects were genotyped for the FAAH polymorphism (rs324420) according to published procedures (42).

**RESULTS**

**Subjects**

Subjects’ demographic information is reported in Table 1. In total, 24 control subjects and 13 active cannabis users were recruited for the study. Three potential cannabis users and two control subjects were excluded or withdrew from the study after screening (for positive drug screens of drugs other than cannabis or relocation). In total, 22 control subjects were matched with 10 cannabis users for age and sex. Education tended to be higher in control subjects, and rate of alcohol use per week was not significantly higher in drug users. Six cannabis users also smoked tobacco (Table 1). Cannabis

| Table 1. Demographic and Clinical Characteristics of Healthy Controls and Cannabis Users |
|-----------------------------------------------|------------------|-----------------|-----------------|-----------------|
| Characteristic                              | Controls ($n = 22$) | Cannabis Users ($n = 10$) | $p$ Value       | $\chi^{2}$     |
| Sex (Females/Males), $n$                     | 11/11            | 3/7             | .29             | 1.1             |
| Age, Years                                  | 34 (11)          | 33 (10)         | .85             | –               |
| Ethnicity (White/Asian/Black), $n$           | 13/7/2           | 7/3/0           | .59             | 1.0             |
| Body Mass Index                             | 23.4 (3.0)       | 24.2 (4.8)      | .58             | –               |
| Genetics (rs324420, C385A), $n$              | 14(CC), 8(AC)    | 7(CC), 2(AC), 1(AA) | .24             | 2.8             |
| Education, Years                            | 16.0 (2.1)       | 14.5 (3.4)      | .14             | –               |
| Alcohol Ever Used, $n$                       | 18               | 10              | .16             | –               |
| Current Alcohol Use/Week                     | 1.0 (2.2)        | 1.9 (2.4)       | .30             | –               |
| Cigarette Smokers, $n$                       | 0                | 6               | <.0001          | –               |
| Cigarettes/Day                               | 0                | 5 (8)           | .003            | –               |
| Barratt Impulsiveness Scale                  |                  |                 |                 |                 |
| Attention                                   | 8.4 (6.0) $^{a}$ | 11.7 (1.3)      | .054            | –               |
| Motor                                       | 15.8 (3.2) $^{a}$ | 16.3 (1.3)      | .63             | –               |
| Self-control                                 | 16.4 (1.9) $^{a}$ | 16.4 (2.2)      | .95             | –               |
| Cognitive Impairment                        | 13.2 (3.6) $^{a}$ | 12.6 (1.3)      | .60             | –               |
| Perseveration                               | 11.4 (1.8) $^{a}$ | 9.2 (1.9)       | .006            | –               |
| Cognitive Instability Impulsiveness          | 7.7 (2.7) $^{a}$ | 6.4 (2.1)       | .19             | –               |
| Attention Impulsivity                        | 14.1 (5.5) $^{a}$ | 18.1 (2.1)      | .036            | –               |
| Motor Impulsivity                            | 25.6 (5.9) $^{a}$ | 25.5 (2.6)      | .98             | –               |
| Non Plan                                     | 27.8 (2.9) $^{a}$ | 29.0 (2.6)      | .30             | –               |
| Marin Apathy Evaluation Scale                | 36.2 (21.6) $^{a}$ | 36.3 (9.8)      | .99             | –               |
| Beck Depression Inventory                    | 1.7 (2.4) $^{a}$ | 5.4 (4.0)       | .004            | –               |
| Amount Injected (mCi)                        | 9.3 (8.8)        | 9.3 (0.8)       | .96             | –               |
| Specific Activity (mCi/µmol)                 | 3270 (1284)      | 3206 (819)      | .88             | –               |
| Mass Injected (µg)                           | 1.0 (0.5)        | 0.9 (0.2)       | .53             | –               |
| Plasma Free Fraction, %                      | 0.81 (0.30)      | 0.70 (0.33)     | .33             | –               |

Values represent mean (SD) unless otherwise indicated.

$^{a}$Data compiled with a subset of healthy controls ($n = 18$).
Table 2. Drug Use Profile of Cannabis Users (n = 10)

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range (Min–Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis Age of Onset, Years</td>
<td>16.1 (3.9)</td>
<td>10–22</td>
</tr>
<tr>
<td>Current Cannabis Use/Week (Grams)</td>
<td>7.5 (4.7)</td>
<td>3.5–14</td>
</tr>
<tr>
<td>Current Cannabis Use/Week (Joints)</td>
<td>20.4 (10.2)</td>
<td>2.3–35</td>
</tr>
<tr>
<td>Years of Cannabis Use</td>
<td>17.5 (10.8)</td>
<td>5–33</td>
</tr>
<tr>
<td>Days Used (Past 90 Days)</td>
<td>76.4 (15.0)</td>
<td>49–90</td>
</tr>
<tr>
<td>Average Cannabis Use/Week (Past 90 Days)</td>
<td>5.9 (1.2)</td>
<td>3.8–7</td>
</tr>
<tr>
<td>Estimated Cannabis Use Days/Yeara</td>
<td>305.6 (60.1)</td>
<td>196–360</td>
</tr>
<tr>
<td>Severity of Dependence Scale</td>
<td>3.0 (2.2)</td>
<td>0–7</td>
</tr>
<tr>
<td>Obsessive Compulsive Smoking Scale</td>
<td>14.2 (7.1)</td>
<td>5–25</td>
</tr>
<tr>
<td>Marijuana Withdrawal Checklist</td>
<td>6.0 (3.9)</td>
<td>1–12</td>
</tr>
<tr>
<td>Marijuana Craving Questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compulsivity</td>
<td>1.4 (0.5)</td>
<td>1–2</td>
</tr>
<tr>
<td>Emotionality</td>
<td>2.9 (1.3)</td>
<td>1–5.3</td>
</tr>
<tr>
<td>Expectancy</td>
<td>4.0 (1.2)</td>
<td>2–6.3</td>
</tr>
<tr>
<td>Purposefulness</td>
<td>4.2 (1.6)</td>
<td>1–6.3</td>
</tr>
<tr>
<td>Total</td>
<td>12.6 (3.9)</td>
<td>5–19.3</td>
</tr>
</tbody>
</table>

aEstimation is based on Time Line Follow Back reported during the past 90 days.

users endorsed more depressive symptoms (Beck Depression Inventory) and greater impulsiveness (BIS) (Table 1). Intake on average was 7.5 ± 4.7 g cannabis/week and had been for 18 ± 11 years (Table 2). No significant differences in [11C]-CURB scan variables were found between groups, including plasma-free fraction of the tracer (Table 1). Groups also were well matched for genotype, such that 70% of controls and cannabis users had the C/C allele for FAAH genetic variant rs324420 (Table 1). Our healthy control data also showed that [11C]CURB binding is not influenced by daytime or the season of scan (Supplemental Figure S1).

Decreased [11C]CURB Binding in Cannabis Users During Early Abstinence

On the basis of our recent finding that brain binding of the FAAH probe [11C]CURB depends on the common genetic polymorphism rs324420 (C385A) (42), genetic variant was used as a covariate in a repeated-measure ANOVA (ROI [12] × group [2]) to investigate differences in \( k_3 \) between controls and cannabis users. This analysis yielded a significant main effect of group \( (F_{1,29} = 6.38, p = .017) \) and genotype \( (F_{1,29} = 11.5, p = .002) \) and a nonsignificant ROI × group interaction \( (F_{4,8,140} = 0.44, p = .82) \). The between-group difference indicated lower brain binding in cannabis users relative to controls, which ranged from −20% (amygdala and cingulate) to −14% (hippocampus) (Figure 1; overall Cohen’s \( d = 0.96 \)). This effect was not accounted for by group differences in use of cigarettes per day \( (F_{1,28} = 5.18, p = .031) \), weekly alcohol consumption \( (F_{1,28} = 8.28, p = .008) \), or global cerebral blood flow \( (F_{1,28} = 4.91, p = .036) \) (according to ASL). Removal of the cannabis user with the only A/A genotype did not change the main effect of group \( (F_{1,28} = 5.25, p = .030) \). Indeed, despite overlap, most of the cannabis users (8–9 of 10) had \( k_3 \) values below the means of the controls (Figure 1 for scatter plots).

Relation Between [11C]CURB Binding and Cannabinoids and Metabolites in Blood and Urine and Trait Impulsiveness

Figure 2 shows cannabinoids and metabolites concentrations in blood and urine at two time points (before and after) on the day of [11C]CURB scan. The blood samples were collected 5.6 ± 0.3 hours apart, whereas urine samples were collected 6.0 ± 0.3 hours apart. Concentrations of CBD, CBN, and THC-gluc in blood and CBD in urine were all below the LOQ. For most subjects (9 of 10), blood THC and 11-OH-THC and urine THC concentrations were below or just above the LOQ and did not show much change between time points. Blood THCCOOH (0–165.5 μg/L) and THCCOOH-gluc (0–665 μg/L) and urine 11-OH-THC (0–77 μg/L) and THCCOOH (14.7–4721 μg/L) concentrations were variable among subjects and showed only slight changes between time points for most subjects. One of 10 subjects had significant blood and urine THC concentrations and had the highest metabolite concentrations that decreased sharply during the 6-hour interval. This subject was also the only one with detectable amount of urine CBN at T1 (Figure 2B), suggesting recent cannabis intake before arrival. Hair drug analyses confirmed THCCOOH presence in 8 of 8 cannabis users with scalp hair available; interestingly, THCCOOH concentrations in hair (0.17–7.0 pg/mg) and in blood and urine were significantly positively correlated with each other (Figure 2C).

We investigated whether low brain [11C]CURB binding in cannabis users correlated with blood and urine THC and metabolite concentrations. Indeed, after excluding the outlier with positive urine CBN at T1, which did not affect the main group effect on [11C]CURB \( k_3 \) \( (F_{1,28} = 5.99, p = .021) \), significant negative correlations were observed between THC or metabolite blood concentrations and \( k_3 \) in amygdala (T1: THCCOOH-gluc, \( r = -.782, p = .013; \) THCCOOH, \( r = -.744, p = .021; \) T2: THCCOOH-gluc, \( r = -.830, p = .006; \) THCCOOH, \( r = -.776, p = .014; \) THC, \( r = -.824, \)
p = .006), medial prefrontal cortex (T2: THCCOOH-gluc, r = −.681, p = .043; THC, r = −.675, p = .046), and anterior cingulate (T1: THCCOOH-gluc, r = −.702, p = .035; T2: THCCOOH-gluc, r = −.710, p = .032). In addition, urine metabolite concentrations correlated with \( \lambda_k \) in amygdala (T1: THCCOOH, r = −.780, p = .014; T2: THCCOOH: r = −.670, p = .048) and hippocampus (T1: THCCOOH, r = −.681, p = .043). Importantly, correlations examined between THC and metabolites and regional \( \lambda_k \) were all negative with different levels of significance, and similar results (not shown) were observed after accounting for C385A genotype (partial correlations). Figure 3 shows examples of correlation plots between amygdala \( \lambda_k \) and blood concentrations of total THC (THC+11-OH-THC), total THCCOOH (THCCOOH+THCCOOH-gluc), and urine THCCOOH.

No significant correlation was observed between self-reported severity of cannabis intake (years of use, days used, joints/gram per week), craving, and withdrawal symptoms (according to Severity of Dependence Scale, Obsessive Compulsive Smoking Scale, Marijuana Withdrawal Checklist, and Marijuana Craving Questionnaire-Short Form) and \( \lambda_k \) values in the ROIs investigated, even after accounting for genetic variability (rs324420). However, we found that \( \lambda_k \) in cannabis users, controlling for rs324420 polymorphism, correlated negatively with impulsiveness (according to the attention factor from BIS; Table 1) across brain regions (r = −.44 to −.77, p = .21 to .014), with significant correlations observed in ventral striatum (r = −.770, p = .014; Figure 4), caudate nucleus (r = −.743, p = .022), medial prefrontal cortex (r = −.711, p = .032), hippocampus (r = −.695, p = .038), prefrontal cortex (r = −.688, p = .040), and temporal cortex (r = −.668, p = .049).

**DISCUSSION**

To our knowledge, this is the first report of differences in levels of the endocannabinoid-metabolizing enzyme FAAH in the living brain of a human with CUD. Contrary to our hypothesis, we found that FAAH binding is significantly lower in the brain of chronic cannabis users during early abstinence and is correlated with chronic and recent cannabis use as evidenced by concentrations of cannabinoids and metabolites in blood and urine and with trait impulsiveness, a CUD behavioral phenotype (55,56).

Our working hypothesis of elevated brain FAAH binding in chronic cannabis users was based on limited, indirect, and sometimes inconsistent data on changes in FAAH and endocannabinoid concentrations. First, studies by Leweke and colleagues (29,30) showed that cerebrospinal fluid AEA concentrations are lower in high- versus low-frequency cannabis users, suggesting perhaps that frequent cannabis intake could increase brain FAAH activities. Second, genetic studies showed that the FAAH variant with higher levels of the enzyme (the C allele) is associated with greater CUD risks and cannabis-related problems [37–41], but see Bidwell **et al.** (55). Echoing these limited findings in humans, preclinical studies showed that mice treated with cannabinoids during adolescence have sustained, markedly increased FAAH protein concentrations in the hippocampus in adulthood (33). In addition, THC-tolerant rats have significantly decreased AEA concentrations in the striatum and midbrain, although opposite findings were
Figure 2. Cannabinoid and metabolite concentrations in (A) blood and (B) urine of chronic cannabis users \(n=10\) collected on arrival \(T_1\) and before discharge \(T_2\) on the day of \(^{11}\text{C}\)CURB positron emission tomography scan and (C) correlations (Pearson) between THCCOOH hair concentrations \(n=8\) and those in blood and urine. The interval between the two time points \(T_1\) and \(T_2\) was approximately 6 hours. Dotted lines identify the limit of quantification (LOQ) for each cannabinoid. Note the one outlier in A and B identified by solid symbols, with the arrow highlighting the only one urine sample positive for cannabinol (CBN). Many values were 0 (below LOQ) for \(\Delta^2\)-tetrahydrocannabinol (THC) (4 and 3), 11-OH-THC (6 and 6), THCCOOH (0 and 1), and THCCOOH-gluc (1 and 1) in blood and THC (8 and 8), 11-OH-THC (2 and 5), and CBN (9 and 10) in urine at \(T_1\) and \(T_2\), respectively, but all urine samples were positive for THCCOOH (14.7–4720.7 ng/mL). THCCOOH, 11-nor-\(\Delta^2\)-carboxy-THC; THCCOOH-gluc, THCCOOH-glucuronide; 11-OH-THC, 11-hydroxy-THC.
observed in the limbic forebrain, and no change was observed in other brain regions, including hippocampus, suggesting possible regionally specific FAAH modulation after chronic CB1 stimulation (31,32).

In our study, rather than the hypothesized elevated FAAH binding, we observed a significant global binding decrease (~14% to 20%; Cohen’s $d = 0.96$) across examined brain regions. This finding was not explained by use of other drugs (nicotine, alcohol, stimulants) or by blood flow (ASL). One possible explanation for our unexpected finding is the presence of residual cannabinoids in brain during the PET scan. It is well known that the highly lipophilic cannabinoids and metabolites have long elimination half-lives in the body because of redistribution and storage in the fat tissue (46–51). For chronic cannabis users compared with occasional users, low but detectable concentrations of THC, 11-OH-THC, and THCCOOH, similar to those observed in our subjects (Figure 2), can be detected in blood (46,48,49) and urine (50) for a prolonged time after the last intake, sometimes as long as 30 to 33 days for THC and THCCOOH (46), which could be related to significant psychomotor impairment documented 3 weeks after last use (61). One study suggested that cannabinoids may accumulate in the brain for even longer than in the blood (62). Currently, no experimental data support that residual cannabinoids may directly occupy FAAH binding sites for $[^{11}C]CURB$. In this regard, although CBD, among many constituents of cannabis, was identified as a moderate (IC$_{50}$ = 15 µmol/L) inhibitor of rodent FAAH (63), a recent study suggested that CBD does not inhibit human FAAH at the same concentrations (64). Nevertheless, residual cannabinoids in the brain may modulate FAAH indirectly, for example, through CB1 activation (65) or through disturbance of the structure and fluidity of lipid membrane where FAAH is located and influencing the activity of the enzyme nonspecifically. Therefore, we cannot exclude the possible confound of residual cannabinoids at the time of PET scan.

In the present study, we measured cannabinoid and metabolite blood and urine concentrations at two time points (approximately 6 hours apart) on the PET day. This was done to disentangle as much as possible new/residual drug exposure from chronic use (47–51). The detection of CBN in one cannabis user at T1 suggests recent cannabis intake (51) despite biochemical verification of smoking abstinence by an
expired carbon monoxide level <10 ppm. This subject also had the highest blood and urine cannabinoid and metabolite concentrations at both time points. Exclusion of this subject did not influence the major finding of decreased FAAH binding in cannabis users. Interestingly, we found a relation between cannabinoid and metabolite concentrations and FAAH binding, suggesting that differences in FAAH binding between groups could be related to recent and chronic cannabis exposure (46,48–50), as opposed to CUD per se. However, our finding that lower FAAH binding is associated with higher impulsiveness (BIS Attention), a known CUD behavioral phenotype (55,56), suggests that low FAAH binding may also predate drug use and be involved in CUD development [but see Tyndale et al. (37)].

A most consistent finding after chronic/repeated cannabinoid exposure in experimental animals and humans is the downregulation of CB1 receptors in the brain that is reversible after prolonged abstinence (19,20,22–25). The possibility of a similar adaptive change in FAAH, an effort to increase AEA in response to low CB1 after chronic cannabis use (and withdrawal), cannot be denied. Cannabis users in our study were scanned at a time that matched the baseline scan of the CB1 report by Hirvonen et al. (20) and had a similar profile of drug use compared with those involved in the two CB1 imaging studies. Exceptions were that daily cannabis intake by our subjects (3 joints) was lower than those in the study by Hirvonen et al. [10 joints (20)] but similar to those in the study by Ceccarini et al. [3 joints (19)] and our subjects were slightly older and had been using cannabis for a longer duration (18 years vs. 10–12 years). Decreased CB1 density at early withdrawal was related to years of cannabis smoking (20) and possibly levels of cannabis consumption (19). In the present study, decreased FAAH binding was not correlated with self-reported drug use, including years or days of use, joints or gram per week, craving, and withdrawal symptoms, possibly in part because self-report has questionable reliability, given widely variable strength of cannabis products and the fact that our subjects were scanned during early withdrawal. However, [11C]Curb jk3 was related to more objective measures of blood and urine cannabinoid and metabolite concentrations, which reflect chronic and recent drug use (46,48–50). It thus appears that decreased FAAH binding and CB1 density during early withdrawal from chronic cannabis use might be related, even though the regional patterns of change are not consistent. Indeed, a recent study showed that enhanced CB1 activity by a gain of function mutation could lead to decreased FAAH expression in the rat brain (65). Future studies to assess FAAH and CB1 in the same cannabis users during early and prolonged abstinence would provide additional insights on regulation of the eCS by chronic cannabis exposure and withdrawal. For example, a dual-tracer PET study can test whether, similar to changes in CB1 receptors, the decreased FAAH binding is reversible after prolonged abstinence and whether CB1 and FAAH are coregulated.

An alternative explanation is that low FAAH binding in cannabis users could be a compensatory adjustment to chronic downregulation of AEA release (29). Further, lower FAAH binding in cannabis users could reflect a suppression of microglial function or even a loss of microglia. In this regard, FAAH is expressed not only in neurons but also in microglia (66,67), the innate immune cells in the brain. Exogenous cannabinoids (by CB2) are known to modulate brain microglia activity and have immunosuppressant effects and could induce apoptosis in immune cells (68,69). In line with our finding, a profound FAAH protein (~80%) and peripheral blood mononuclear cell (T, B, and natural killer) loss was observed after chronic (6–36 months) daily cannabis intake in a group of high school and university students (70).

Limitations of the study include unequal sample size in the case-control study, a small sample size for cannabis users, and more tobacco smokers in cannabis users than in control subjects. Nevertheless, the fact that the finding of our study was opposite to our hypothesis highlights our limited knowledge about FAAH and endocannabinoid regulation, especially in humans. Currently, a clinical trial with the Pfizer FAAH inhibitor PF-04457845 (71–73) is ongoing for cannabis withdrawal (NCT01618656 at ClinicalTrials.gov). Our recent human blocking study with the Pfizer compound and [11C]Curb PET provided crucial information on the dosing regimen that produces sufficient FAAH inhibition in the human brain (44).

The current finding of decreased FAAH binding, together with literature findings of downregulated CB1 function (19,20,25) and decreased AEA concentrations in cerebrospinal fluid of heavy cannabis users (23), suggests that brain endogenous cannabinoid activities could be suppressed by chronic exposure to cannabis, which might underlie at least in part the many cannabis withdrawal effects, given the important roles of endocannabinoids in modulating many neurotransmitter functions and the report that higher cerebrospinal fluid AEA levels are associated with lower risks of psychotic symptoms after cannabis use (29). For now, our finding on FAAH status in cannabis withdrawal is still preliminary, and no data are available on FAAH changes during the course of cannabis withdrawal and its relation to endocrine function [e.g., glucocorticoids, corticotropin-releasing factor (74,75)] and neuroinflammation. If decreased FAAH binding represents a compensatory rescuing effort of the brain to maintain endocannabinoid activity, further inhibition with drugs such as the Pfizer compound might help boost the effect. In this scenario, it might be worth emphasizing that a partial (25%–50%) FAAH loss, as evidenced by rs324420 genetics (42) and in contrast to a current trial approach that aimed at full shutdown of FAAH (72,76), is functionally significant in at least some aspects of human behaviors, for example, mood (77), and treatment development should take this into consideration.

In conclusion, to our knowledge, we report for the first time that FAAH binding is decreased in the brain of chronic cannabis users during early withdrawal and that this is related to chronic and recent cannabis intake and to trait impulsiveness. This should be considered when developing novel FAAH- and endocannabinoid-targeting strategies for CUD.

ACKNOWLEDGMENTS AND DISCLOSURES
This work was supported in part by Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation (SH), the National Institute of Health and National Institute of Drug Abuse NIH/NIDA Grant No. R21 DA036024 (IB), Canadian Institutes of Health Research Grant No. TMI109787 (RFT), and an Endowed Chair in Addictions (Psychiatry Department, RFT).

All authors report no biomedical financial interests or potential conflicts of interest.
FAAH and Cannabis Use Disorder

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Received Jan 5, 2016; revised Mar 24, 2016; accepted Apr 18, 2016. Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2016.04.012.

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