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# Brain imaging of cannabinoid type 1 (CB<sub>1</sub>) receptors in women with cannabis use disorder and male and female healthy controls

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## Abstract

Cannabis effects are predominantly mediated by pharmacological actions on cannabinoid type 1 (CB<sub>1</sub>) receptors. Prior positron emission tomography (PET) studies in individuals who use cannabis included almost exclusively males. PET studies in females are needed because there are sex differences in cannabis effects, progression to cannabis use disorder (CUD), and withdrawal symptom severity. Females with CUD ( $N = 10$ ) completed two double-blind cannabis smoking sessions (Session 1: placebo; Session 2: active), and acute cannabis effects were assessed. After Session 2, participants underwent 3 days of monitored cannabis abstinence; mood, craving, and withdrawal symptoms were assessed and a PET scan (radiotracer: [<sup>11</sup>C]OMAR) followed. [<sup>11</sup>C]OMAR Distribution volume ( $V_T$ ) from these participants was compared with  $V_T$  of age/BMI-similar female non-users of cannabis ("healthy controls";  $N = 10$ ).  $V_T$  was also compared between female and male healthy controls ( $N = 7$ ). Females with CUD displayed significantly lower  $V_T$  than female healthy controls in specific brain regions (hippocampus, amygdala, cingulate, and insula). Amygdala  $V_T$  was negatively correlated with mood changes (anger/hostility) during abstinence, but  $V_T$  was not correlated with other withdrawal symptoms or cannabis effects. Among healthy controls, females had significantly higher  $V_T$  than males in all brain regions examined. Chronic cannabis use appears to foster downregulation of CB<sub>1</sub> receptors in women, as observed previously in men, and there are inherent sex differences in CB<sub>1</sub> availability. Future studies should elucidate the time course of CB<sub>1</sub> downregulation among females who use cannabis and examine the relation between CB<sub>1</sub> availability and cannabis effects among other populations (e.g., infrequent users; medicinal users).

## KEYWORDS

cannabis use disorder, PET imaging, sex differences

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## 1 | INTRODUCTION

Cannabis use has increased in recent years as legalization of the drug has expanded. In the United States, where cannabis is now legal for medicinal purposes in 35 states and non-medicinal (“recreational”) purposes in 15 states, the percentage of adults reporting past-year cannabis use increased from 10.4% in 2002 to 17.5% in 2019.<sup>1,2</sup> One negative consequence of chronic cannabis use is the potential for development of cannabis use disorder (CUD).<sup>3–5</sup> Individuals with CUD may experience various problems related to their cannabis use such as an inability to stop using despite medical or psychosocial problems, development of tolerance, and emergence of withdrawal symptoms upon abstinence.<sup>3</sup> Given that in the United States alone, approximately 6.4 million people have CUD,<sup>2</sup> further understanding of the neurological consequences associated with regular cannabis use is needed.

The endocannabinoid system is composed of two main G protein-coupled receptors: cannabinoid type 1 (CB<sub>1</sub>) and type 2 (CB<sub>2</sub>). The two main endogenous ligands that bind to, and activate, these receptor subtypes are *N*-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG).<sup>6,7</sup> The primary psychoactive constituent of cannabis ( $\Delta^9$ -tetrahydrocannabinol; THC) is a partial agonist at CB<sub>1</sub> and CB<sub>2</sub> receptors<sup>8</sup> and is believed to be the main driver of the hallmark effects (e.g., subjective “high,” paranoia, and cognitive impairment) synonymous with using THC-dominant cannabis.<sup>9–12</sup> Cannabis/THC produces such a wide range of acute effects because CB<sub>1</sub> receptors are located in high density in many brain regions, including those involved in drug reward, mood, motor functioning, learning, and higher-order cognition.<sup>13–17</sup>

In prior positron emission tomography (PET) studies,<sup>15,16,18</sup> individuals who used cannabis chronically displayed significantly lower CB<sub>1</sub> receptor availability than healthy controls (i.e., non-users of cannabis), suggesting repeated cannabis use produces CB<sub>1</sub> down-regulation. These studies are limited, however, because they included nearly all male participants; of the three prior applicable studies, two<sup>15,18</sup> included exclusively males, and the other<sup>16</sup> only included two females. Importantly, key sex differences have been observed following both acute and long-term cannabis exposure. First, females are more sensitive to acute cannabis effects than males. After smoking cannabis, females report higher ratings on subjective items of abuse liability (e.g., “good drug effect”) than males matched for cannabis-use characteristics.<sup>19</sup> Among individuals who use cannabis infrequently, women report stronger negative/adverse cannabis effects (e.g., “anxious/nervous”) than men.<sup>20</sup> In another study,<sup>21</sup> females and males smoked a cannabis joint ad libitum; females experienced similar magnitude of pharmacodynamic effects as males, despite the fact that they smoked significantly less and were exposed to less THC than males. Second, upon abstinence from regular cannabis use, females exhibit more severe withdrawal symptoms than males<sup>22,23</sup>; moreover, CUD reduces self-reported quality of life to a greater extent in females versus males.<sup>24</sup> Finally, relative to men, women show an accelerated trajectory from their first use of cannabis to the development of CUD (i.e., a “telescoping effect”).<sup>25,26</sup> Taken together, these

findings underscore the need for neuroimaging studies with female participants to elucidate potential neuroadaptations of CB<sub>1</sub> receptors related to chronic cannabis use and to examine whether individual-level characteristics (e.g., acute drug effects and magnitude of withdrawal) are related to CB<sub>1</sub> receptor availability.

This study extends prior PET studies that included predominantly male participants by comparing CB<sub>1</sub> receptor availability in females with CUD to that of female healthy controls (i.e., non-users). Females with CUD completed two cannabis self-administration sessions and a monitored 3-day period of cannabis abstinence, and we explored the relation between acute cannabis effects and mood/craving/withdrawal and CB<sub>1</sub> receptor availability. The length of the inpatient stay was set at 3 days based on prior studies that have shown that cannabis withdrawal symptoms typically emerge and peak within the first 3 days after cessation.<sup>27,28</sup> We hypothesized that females with CUD would show lower CB<sub>1</sub> availability compared with female controls. We also hypothesized that CB<sub>1</sub> availability in regions associated with mood regulation (i.e., amygdala and hippocampus) would be correlated with negative mood, craving, and withdrawal symptoms during cannabis abstinence, while CB<sub>1</sub> availability in regions associated with reward, cognition, and/or motor functioning (i.e., cingulate, frontal cortex, putamen, and ventral striatum) would be correlated with acute pharmacodynamic effects of cannabis.

As a secondary aim, we compared CB<sub>1</sub> availability in male and female healthy controls to explore potential CB<sub>1</sub> sex differences, unrelated to cannabis use. Preliminary studies of non-cannabis users have detected differences in CB<sub>1</sub> availability between men and women, but have opposing results; one study showed higher CB<sub>1</sub> availability in men,<sup>29</sup> and two others<sup>14,30</sup> showed higher CB<sub>1</sub> availability in women. We extend these studies by imaging female participants in the same menstrual cycle phase (follicular) to minimize the influence of hormonal changes on radiotracer binding to CB<sub>1</sub> receptors.

## 2 | METHODS

The present study was conducted at the Johns Hopkins University (JHU) Behavioral Pharmacology Research Unit, the JHU Clinical Research Unit (CRU), and the JHU Hospital PET Imaging Center. The JHU School of Medicine Institutional Review Board approved all study procedures that were conducted in accordance with the Declaration of Helsinki. The study was registered on ClinicalTrials.gov (NCT03204305).

### 2.1 | Participants

This report describes data for three sets of participants: (1) women with CUD; (2) contemporary female healthy controls; (3) female and male historical healthy controls. All participants provided written informed consent prior to participation.

## 2.1.1 | Females with CUD and contemporary female controls

Healthy female volunteers (18–50 years old) who used cannabis or did not use cannabis were recruited from the Baltimore metropolitan area using online and print media advertisements. Potential study candidates first completed a brief telephone screen and those who appeared eligible were invited for an in-person screening visit where they were interviewed using a battery of diagnostic and psychological instruments to determine study eligibility. Recent drug/alcohol use was assessed via the 90-day Timeline Follow-Back<sup>31</sup> and participants also completed the Mini-International Neuropsychiatric Interview (MINI)<sup>32</sup> for DSM-V (MINI, v.7) to determine whether they met criteria for any psychiatric conditions or substance use disorders. Candidates also completed a medical assessment that included a medical history review, an electrocardiogram (EKG), routine blood testing (chemistry, haematology, and serology), urine drug toxicology and pregnancy tests, breath alcohol and exhaled CO tests, and a physical examination. Participants were also trained on cognitive performance tasks (see below) at the screening session until they reached a stable baseline to avoid practice effects during the drug administration sessions.

Primary inclusion criteria for women who used cannabis were use of cannabis, on average,  $\geq 25$  days per month in the past 3 months; positive urine specimen for cannabis; meet DSM-V criteria for moderate or severe CUD; and report  $\geq 2$  cannabis withdrawal symptoms during a past cannabis abstinence period. For healthy contemporary controls, primary inclusion criteria were no cannabis use for  $>12$  months;  $\leq 5$  lifetime cannabis uses; and negative urine test for cannabis.

Primary exclusion criteria for all contemporary participants included psychiatric or substance use disorder (aside from CUD for cannabis users); serious medical condition; medication use that may impact participant safety or interfere with study outcomes (e.g., hormonal contraceptives); seeking treatment for cannabis-related problems; unstable hypertension; positive test for pregnancy or illicit drugs (aside from cannabis for women with CUD); past-year radiation exposure that would result in cumulative exposure of  $\geq 5$  rem after study completion; and clinically significant EKG or incidental MRI finding.

## 2.1.2 | Historical male and female healthy controls

Historical healthy controls who did not use cannabis were selected from datasets for prior [<sup>11</sup>C]OMAR studies of males<sup>13</sup> and females (Klarman Family Foundation, unpublished). Historical controls were selected to match age and BMI ranges for women with CUD from the current study.

Historical controls met similar inclusion/exclusion criteria: good health (e.g., medical history, lab results, and physical examination); no psychiatric or substance-use disorders; no self-reported drug use or heavy alcohol use in the last 90 days; and negative urine test for common drugs of abuse (e.g., amphetamine, cocaine, opiates, and cannabis).

## 2.2 | Procedures

### 2.2.1 | Cannabis administration sessions

Cannabis users completed two acute drug administration sessions in which they smoked cannabis, via a hand-held pipe, containing either  $\sim 0$  mg THC or 25 mg THC; participants and research staff were blinded to THC dose. Active and placebo cannabis was obtained from the National Institute on Drug Abuse (NIDA) Drug supply program. Cannabis was stored in a freezer and rehydrated (for at least 24 h) before being administered to participants. Sessions were completed in a fixed order (session 1: placebo; session 2: active) so that the time since last cannabis use would be consistent for all participants during subsequent procedures. Active cannabis had a high concentration of THC (13.4%) and a low concentration ( $<1\%$ ) of cannabidiol (CBD) and cannabinol (CBN). Placebo cannabis contained  $<0.01\%$  THC and did not contain detectable levels of CBD or CBN. The quantity of plant material in the pipe was the same for placebo and active sessions (186.6 mg). After each drug administration, research staff returned the pipe to BPRU pharmacy staff who confirmed, via visual inspection, that all plant material had turned to ash (this indicated that the full dose was administered).

Prior to drug administration in each session, participants were fed a standard low-fat breakfast ( $\sim 300$  calories), had an IV catheter placed in their forearm vein, had a baseline blood sample collected, and completed baseline pharmacodynamic measures (i.e., cognitive performance, subjective effects, and vital signs). Blood samples were collected and pharmacodynamic measures were completed periodically (i.e., 15- to 30-min intervals) for 3 h following cannabis administration. During each session, participants were instructed to smoke the entire contents of the pipe (which was pre-loaded with either placebo or active cannabis by BPRU pharmacy staff) *ad libitum* within a 10-min period; the pipe was fitted with a metal top to obstruct the view of the plant material and preserve the study blind, as active and placebo cannabis may differ in color.

### 2.2.2 | Inpatient period

Immediately following session 2, females with CUD were admitted to an inpatient facility (the CRU) for 3 days. During this time, participants were medically monitored and could not use cannabis or any other drugs and were not permitted to have visitors. Mood, craving, and withdrawal symptoms were assessed twice daily (see below).

### 2.2.3 | Magnetic resonance imaging (MRI)

Prior to their PET scan, all participants completed a volumetric MRI scan, on a 3-T Prisma scanner, using a spoiled GRASS (gradient recalled acquisition in steady state) SPGR sequence. The purpose of the MRI scan was to enable sampling of PET data in specific brain regions of interest (or volumes of interest; VOI) for each individual participant (see below).

## 2.2.4 | Positron emission tomography (PET) scan

PET scans were completed on a Siemens ECAT HRRT brain-dedicated high resolution scanner (Siemens Healthcare, Knoxville, TN), which has expected resolutions of less than 2.5 mm in three directions.<sup>33</sup> The radiotracer used was [<sup>11</sup>C]OMAR. Women with CUD completed their scan after 3 days of monitored cannabis abstinence, while healthy controls completed their scan during an outpatient visit. A facemask was custom-made for each participant to minimize head movement during the scan. An intravenous catheter was inserted into a forearm vein to allow for injection of the radioligand. An arterial catheter was also inserted into the radial artery at the wrist, on the hand opposite the intravenous catheter, to allow for repeated blood sampling. Following a 6-min attenuation scan, a slow bolus dose (over 1 min) of [<sup>11</sup>C]OMAR (18–20 millicurie, mCi) was administered intravenously and acquisition of dynamic PET data in 3D list mode commenced, which lasted 90 min. Specific activity and mass of [<sup>11</sup>C]OMAR were comparable across participants (see Table S1).

For approximately the first 5 min of the scan, arterial blood samples (~2 ml) were collected rapidly (every 5 s). Larger blood samples (~5 ml) were collected at 5, 10, 30, 60, and 90 min.

PET scans were reconstructed using the iterative ordered-subset expectation-maximization (OSEM) algorithm, which corrected for normalization, attenuation, scatter, random, and dead-time.<sup>34</sup> The frame schedule used was as follows: four 15-s, four 30-s, three 1-min, two 2-min, five 4-min, and 12 5-min frames. This resulted in a total of 30 frames for the 90-min scan. Each PET frame consisted of 256 (left-to-right) by 256 (nasion-to-inion) by 207 (neck-to-cranium) of cubic voxels (1.27 mm).

Radioactivity concentrations of [<sup>11</sup>C]OMAR in plasma were measured with a cross-calibrated gamma counter and corrected for physical decay relative to the tracer injection time. Fractions of radioactive metabolites in plasma were quantified using high performance liquid chromatography (HPLC).<sup>13</sup> Time-radioactivity curves (TACs) of metabolite-corrected fractions were obtained by interpolating parent fractions from HPLC at blood sampling times using the shape-preserving piecewise cubic interpolation function of Matlab (MathWorks, Natick, Massachusetts).

## 2.2.5 | Additional procedures for female participants

At screening, all women (including historical controls) provided details on their recent menstrual history. Upon enrolment, women were instructed to track their menstrual cycles and to report their first day of menstruation to staff. PET scan dates were scheduled within the first 12 days of their menstrual phase to target the follicular phase. Blood samples were collected on the day of the PET scan for analysis of plasma progesterone to verify they were in the follicular phase; all females (i.e., contemporary and historical participants) were confirmed to be in their follicular phase at the time of their scan based on blood progesterone concentrations being <1.0 ng/ml.

## 2.3 | Outcome measures

### 2.3.1 | Cannabis administration sessions

Pharmacodynamic outcomes measured during the two cannabis administration sessions included (1) subjective drug effects, assessed with a 21-item Drug Effect Questionnaire (DEQ) that consisted of items reflective of positive/reinforcing drug effects (e.g., “like drug effect”), negative/aversive effects (e.g., “unpleasant drug effect”), and mood states related specifically to cannabis intoxication (e.g., “paranoid” and “hungry/have munchies”).<sup>11,12</sup> Items were presented individually on a 100-mm visual analog scale; (2) cognitive and psychomotor performance, assessed with three computerized tasks previously shown to be sensitive to cannabis intoxication: the Digit Symbol Substitution Task (DSST),<sup>35</sup> the Divided Attention Task (DAT),<sup>36</sup> and the Paced Auditory Serial Addition Task (PASAT)<sup>37</sup>; for detailed descriptions of these tasks, see prior studies,<sup>11,12</sup> and (3) vital signs (i.e., heart rate and systolic/diastolic blood pressure). Blood samples were collected in each session and analysed (via LC-MS/MS<sup>11,38,39</sup>) for concentrations of THC and its two primary metabolites (11-OH-THC and THCCOOH).

### 2.3.2 | Inpatient period

Each day during the inpatient stay, women with CUD completed a series of psychological self-report instruments including the Marijuana Withdrawal Checklist (MWC),<sup>27</sup> the Marijuana Craving Questionnaire Short Form (MCQ-SF),<sup>40</sup> and the Profile of Mood States (POMS)-2 Adult Short Form<sup>41,42</sup>; these were also all given prior to sessions 1 and 2 (when participants were using cannabis as usual).

On the MWC, participants rated the severity of 32 symptoms on a scale of 0 (not at all) to 3 (severe); several items were non-specific in nature to minimize response bias. Items assessed a combination of mood (e.g., “depressed mood” and “nervousness/anxiety”) and physiological symptoms (e.g., “sweating,” “stomach pains,” and “dizziness”) and collectively yielded a composite withdrawal discomfort score. On the MCQ-SF, cannabis-related craving was assessed with 14 items that were reflective of four distinct dimensions of craving (i.e., compulsivity, emotionality, expectancy, and purposefulness). The POMS-2-Short evaluates six distinct dimensions of mood (i.e., Tension/Anxiety, Anger/Hostility, Vigour/Activity, Fatigue/Inertia, Depression/Dejection, and Confusion/Bewilderment) using 35 separate subjective items (five of the items are “dummy items” that are not incorporated into overall domain scores). In addition to the individual subscales, the POMS-2-Short also yields a total mood disturbance score.

### 2.3.3 | Volumes of interest (VOIs)

MRIs were submitted to Freesurfer software version 6.0<sup>43</sup> (<http://surfer.nmr.mgh.harvard.edu>) for automated parcellation of

brain regions. We focused on eight VOIs (ventral striatum, amygdala, putamen, cingulate, globus pallidus, insula, frontal cortex, and hippocampus); these brain regions were chosen a priori because they are associated with drug reward, mood, cognition, motor function, and/or habit learning. The frontal cortex VOI included original Freesurfer VOIs belonging to the frontal cortex.<sup>44</sup> The VOIs were transferred to PET space by utilizing MRI-to-PET co-registration parameters, using the co-registration module of SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) and applied on PET frames to obtain TACs for each VOI.

### 2.3.4 | [<sup>11</sup>C]OMAR distribution volume ( $V_T$ )

$V_T$  values of individual regions were obtained by plasma-reference graphical analysis (PRGA).<sup>13,45</sup> A composite  $V_T$  was calculated as weighted means of original Freesurfer VOIs of the above eight regions (minus the ventral striatum) and the cerebellum, anterior and posterior cingulate cortices, occipital, parietal and temporal cortices, and thalamus, to closely approximate the composite regions defined by D'Souza et al.<sup>15</sup>

## 2.4 | Data analysis

Peak change-from-baseline pharmacodynamic data from the cannabis administration sessions were analysed using linear mixed models. These analyses included the 14 participants who completed the active and placebo sessions. Separate analyses were conducted for each outcome; cannabis dose (active vs. placebo) was the lone factor in the models, and the covariance structure used was compound symmetry (CS). Bonferroni corrections were made to reduce family-wise error rate.

For the MWC, MCQ-SF, and POMS-2-Short, paired samples *t* tests compared baseline scores (i.e., when participants were using cannabis as usual before the second cannabis smoking session) to peak scores observed during cannabis abstinence. Bonferroni corrections were made to reduce family-wise error rate.

$V_T$  (in each VOI) was compared between: (1) females with CUD who completed the PET scan ( $N = 10$ ) and female healthy controls ( $N = 10$ , two contemporary and eight historical controls) and (2) female and male healthy controls ( $N = 7$ ), using the following approach: a robust linear regression model with group status (e.g., CUD vs. control) encoded as a dummy variable, age as a covariate, and  $V_T$  for the eight VOIs as the dependent variables, followed by post hoc comparisons (Tukey's HSD) between groups. Composite  $V_T$  was compared between the same groups using independent samples *t*-tests.

Correlations (Spearman's  $\rho$ ) explored relations between: (1)  $V_T$  in the amygdala, hippocampus, and composite  $V_T$  and severity of cannabis withdrawal symptoms, mood disturbance, and craving during the inpatient stay (peak-change-from-baseline) and (2)  $V_T$  in the ventral striatum, cingulate, frontal cortex, putamen, and composite  $V_T$  and positive subjective effects and cognitive performance (peak-change-from-baseline) following active cannabis administration. These specific correlations were hypothesis-driven and decided on a priori (see Section 1). Significance was set at  $p < 0.05$  for all analyses. Bonferroni corrections were made to reduce family-wise error rate.

Statistical parametric mapping (SPM) tested for  $V_T$  differences between groups of interest (i.e., females with CUD vs. female healthy controls and male vs. female healthy controls), without restriction of VOIs.  $V_T$  maps were generated by applying PRGA to PET voxels. MRIs were spatially normalized to Montreal Neurological Institute (MNI) space using SPM-supplied probabilistic templates.  $V_T$  maps were

**TABLE 1** Demographic characteristics

Characteristic	Mean (SD) or number		
	Females with CUD ( $N = 10$ )	Female HC ( $N = 10$ )	Male HC ( $N = 7$ )
Age (years)	23.2 (2.7)	25.5 (5.0)	29.6 (6.9)
BMI ( $\text{kg}/\text{m}^2$ )	25.7 (6.0)	23.6 (4.3)	24.4 (2.1)
Race: Caucasian	4	9	1
Race: Black/AA	5	1	5
Race: Other or unknown	1	0	1
Cigarette smokers	1 <sup>a</sup>	1 <sup>b</sup>	0
Grams cannabis used/day	4.8 (3.0)	N/A	N/A
Days of cannabis use in past 90	83.6 (10.4)	N/A	N/A
Age of first cannabis use	15.3 (3.0)	N/A	N/A
Years of cannabis use	7.9 (4.3)	N/A	N/A
Moderate cannabis dependence	7	N/A	N/A
Severe cannabis dependence	3	N/A	N/A

Note: Two of 10 female HC were contemporary participants while remaining eight were historical controls. BMI data missing for two male healthy controls.

<sup>a</sup>Smoked two cigarettes/day.

<sup>b</sup>Smoked three cigarettes/week.

spatially normalized by applying parameters of PET-to-MRI co-registration and MRI spatial normalization parameters and smoothed by a Gaussian kernel of 8 mm (full-width at half-maximum, FWHM). Clusters are reported when the extent threshold exceeded  $p < 0.05$ , false-discovery-rate (FDR)-corrected, while setting height threshold at  $p < 0.005$ , uncorrected.

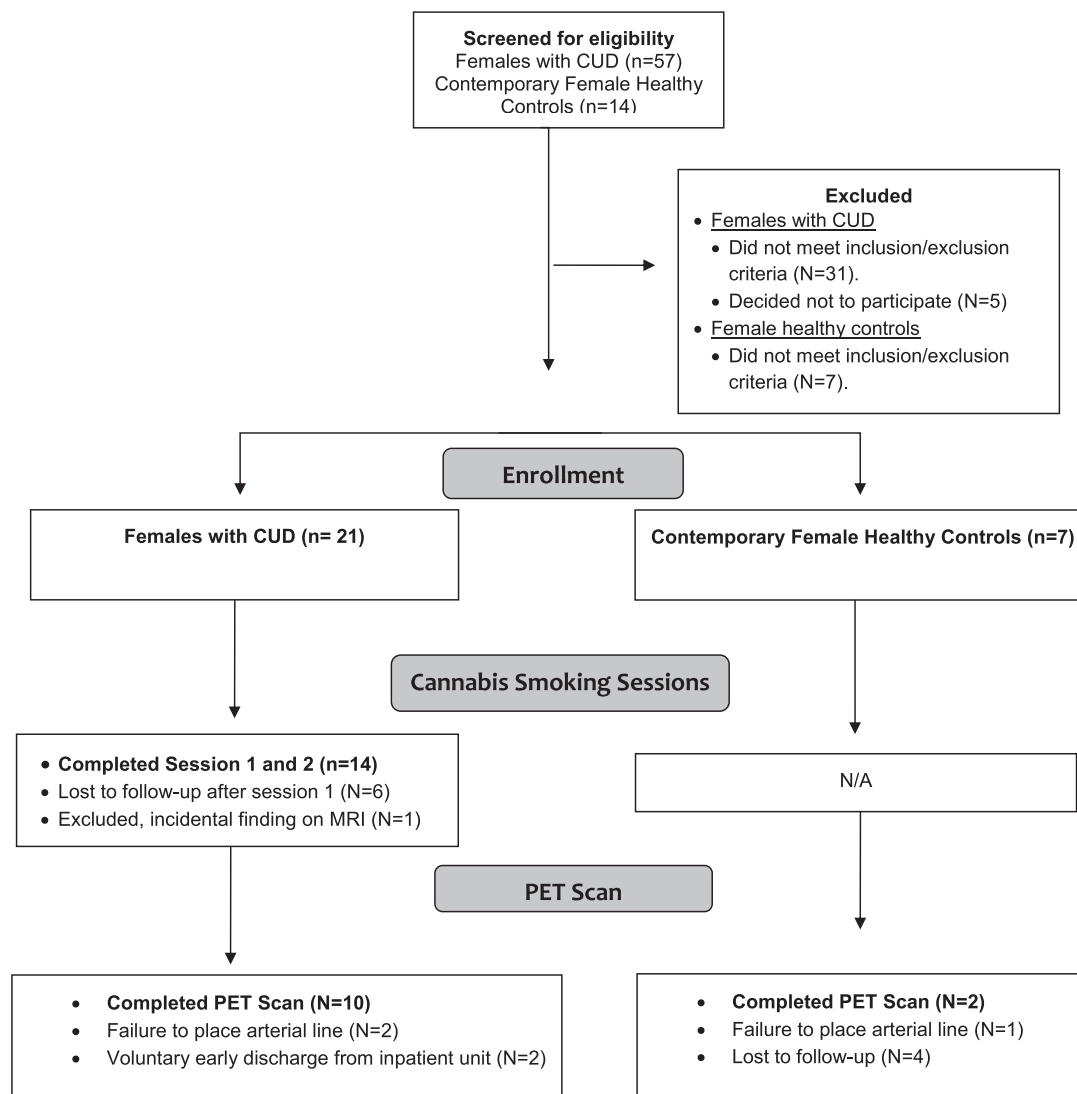
### 3 | RESULTS

#### 3.1 | Participants

Table 1 depicts participant demographic characteristics. BMI and age were compared across groups of interest using independent samples  $t$  tests (i.e., females with CUD vs. female healthy controls; male vs. female healthy controls). Neither BMI or age differed significantly across these groups. Figure 1 (CONSORT diagram) shows the participation flow of females with CUD and contemporary controls.

#### 3.2 | Cannabis administration session data

Figure 2 depicts data from cannabis administration sessions. Participants ( $N = 14$ ) reported higher ratings for “Drug Effect” ( $F = 31.32$ ,  $p < 0.001$ ), “Pleasant” ( $F = 21.10$ ,  $p < 0.001$ ), “Like” ( $F = 19.16$ ,  $p < 0.001$ ), and “Throat Irritated” ( $F = 5.15$ ,  $p = 0.04$ ) in the active session relative to placebo; “Drug Effect” ( $F = 31.32$ ,  $p = 0.001$ ), “Pleasant” ( $F = 21.10$ ,  $p = 0.01$ ), and “Like” ( $F = 19.16$ ,  $p = 0.02$ ) each remained significant after the Bonferroni correction was applied, but “Throat Irritated” did not survive the alpha correction. DSST performance was worse in the active, compared with placebo condition ( $F = 9.0$ ,  $p = 0.01$ ), though this result did not remain significant after applying the alpha correction. DAT and PASAT performance did not differ between sessions. Heart rate increased significantly ( $F = 26.79$ ,  $p < 0.001$ ) after active cannabis administration relative to placebo, and this finding remained significant with the Bonferroni correction ( $F = 26.79$ ,  $p = 0.001$ ) (see Table S2 for full results for pharmacodynamic data).



**FIGURE 1** CONSORT diagram. N/A = not applicable for that participant group



### 3.3 | Inpatient abstinence data

POMS-2-Short scores for Depression/Dejection" ( $t = -2.86$ ,  $p = 0.02$ ), Tension/Anxiety ( $t = -3.28$ ,  $p = 0.01$ ), and Vigour/Activity ( $t = -10.58$ ,  $p < 0.001$ ) were elevated during cannabis abstinence relative to baseline (i.e., cannabis use as usual); however, once the Bonferroni alpha corrections were applied, only Vigour/Activity remained significant ( $t = -10.58$ ,  $p < 0.001$ ). MWC composite withdrawal discomfort score ( $t = -2.52$ ,  $p = 0.03$ ) and MCQ-SF compulsivity ( $t = -2.51$ ,  $p = 0.03$ ) and expectancy scores ( $t = -2.41$ ,  $p = 0.04$ ) increased during cannabis abstinence relative to baseline, but the significant findings on the MCQ-SF did not survive the alpha correction (see Table S3 for full results for inpatient cannabis abstinence data).

### 3.4 | [ $^{11}\text{C}$ ]OMAR distribution volume ( $V_T$ ) comparisons

As shown in Figure 3, females with CUD displayed lower  $V_T$  than female healthy controls in the amygdala (estimated difference,  $ED = -0.28 \pm 0.11$ ,  $t = -2.51$ ,  $p = 0.02$ ), cingulate ( $ED = -0.25 \pm 0.11$ ,  $t = -2.36$ ,  $p = 0.03$ ), hippocampus ( $ED = -0.27 \pm 0.11$ ,  $t = -2.35$ ,  $p = 0.03$ ), and insula ( $ED = -0.25 \pm 0.11$ ,  $t = -2.23$ ,  $p = 0.04$ );  $V_T$  in the other four VOIs (ventral striatum, putamen, globus pallidus, and frontal cortex) and composite  $V_T$  did not differ significantly between these two groups.

As shown in Figure 4, female healthy controls displayed higher  $V_T$  than male healthy controls in the amygdala ( $ED = 0.40 \pm 0.12$ ,  $t = 3.22$ ,  $p < 0.01$ ), cingulate ( $ED = 0.37 \pm 0.12$ ,  $t = 3.20$ ,  $p < 0.01$ ), frontal cortex ( $ED = 0.33 \pm 0.11$ ,  $t = 2.97$ ,  $p < 0.01$ ), globus pallidus ( $ED = 0.49 \pm 0.15$ ,  $t = 3.18$ ,  $p < 0.01$ ), hippocampus ( $ED = 0.35 \pm 0.13$ ,  $t = 2.76$ ,  $p = 0.01$ ), insula ( $ED = 0.38 \pm 0.12$ ,  $t = 3.10$ ,  $p < 0.01$ ), putamen ( $ED = 0.39 \pm 0.13$ ,  $t = 3.10$ ,  $p < 0.01$ ), and ventral striatum ( $ED = 0.31 \pm 0.12$ ,  $t = 2.68$ ,  $p = 0.02$ ). Composite  $V_T$  was higher in female versus male controls ( $t = 2.95$ ,  $p = 0.01$ ).

### 3.5 | Correlations between $V_T$ and behavioral outcomes among females with CUD

$V_T$  in the amygdala and hippocampus were negatively correlated with peak-change-from-baseline scores for Anger/Hostility on the POMS-2-Short (amygdala:  $\rho = -0.79$ ,  $p = 0.007$ ; hippocampus:  $\rho = -0.69$ ,  $p = 0.03$ ). Peak-change-from-baseline scores for MCQ-SF Factor 4 (purposefulness) were positively correlated with hippocampus  $V_T$  ( $\rho = 0.73$ ,  $p = 0.02$ ). Once the Bonferroni alpha corrections were applied, the correlation between  $V_T$  in the amygdala and Anger/Hostility on POMS-2-Short remained significant ( $\rho = -0.79$ ,  $p = 0.047$ ), but the other two significant correlations noted above did not survive the alpha correction (see Table S4). There were no other significant correlations between amygdala and hippocampus  $V_T$  (nor composite  $V_T$ ) and inpatient data.

There were no significant correlations between  $V_T$  in the ventral striatum, cingulate, frontal cortex, and putamen (nor composite  $V_T$ ) and pharmacodynamic effects including positive subjective effects and cognitive performance (see Table S4 for full correlation results).

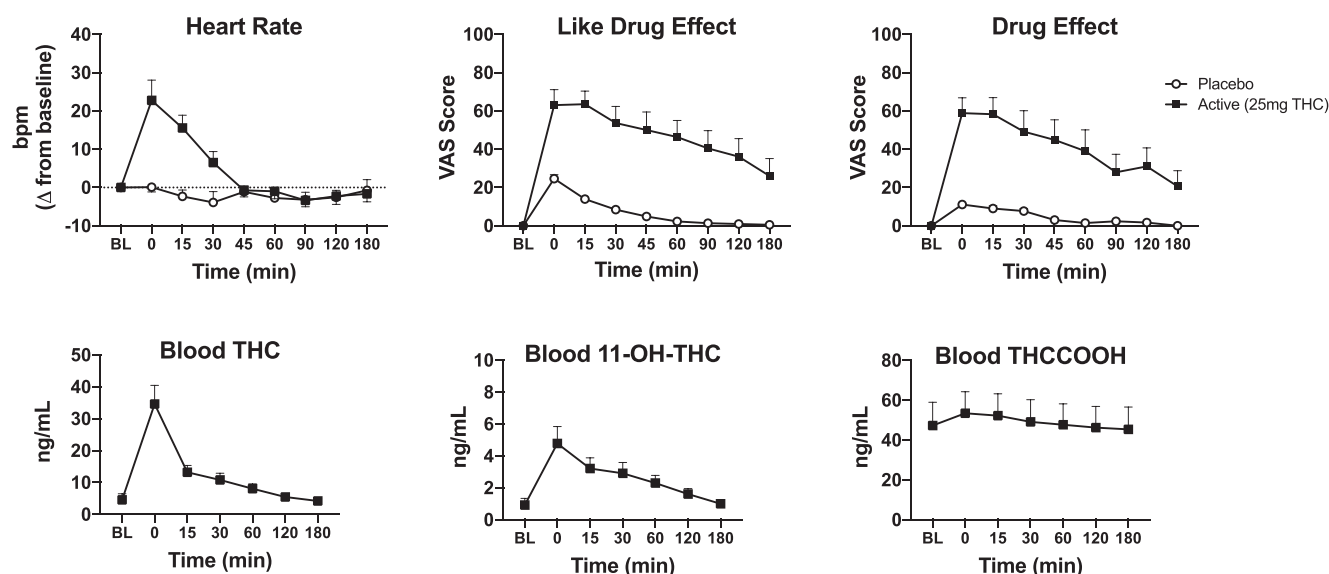
### 3.6 | SPM voxel-wise $V_T$ comparisons

Female healthy controls showed extended areas of greater  $V_T$  compared with male healthy controls ( $p < 0.001$ , FDR-corrected at cluster level;  $k_E = 96\,478$  (777.2 ml). The peak T (7.09) was observed (at  $-36$ ,  $-32$ , and  $42$ ) in the left postcentral gyrus (see Figure S1). No above-threshold clusters were observed between females with CUD and female controls.

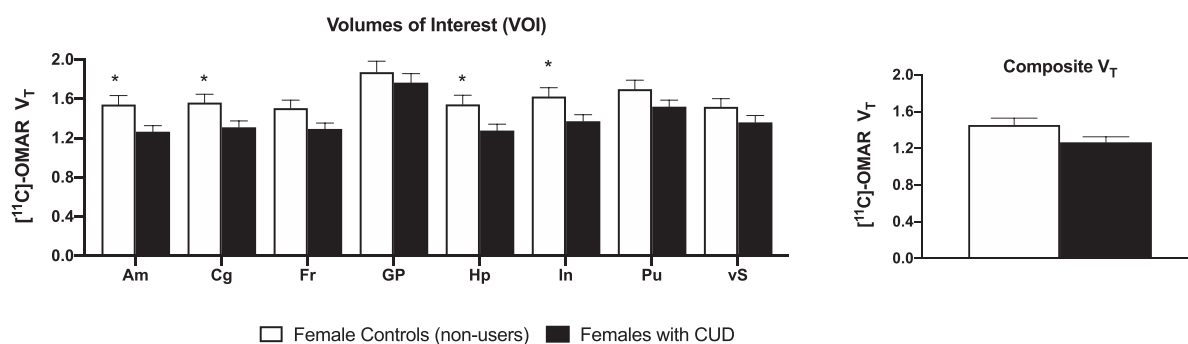
## 4 | DISCUSSION

Females with CUD displayed 16–18% lower  $V_T$ , on average, in the amygdala, cingulate, hippocampus, and insula after 3 days of monitored cannabis abstinence when compared with female healthy controls. In three prior  $\text{CB}_1$  PET studies with predominantly male participants,<sup>15,16,18</sup> comparable (or lower) reductions in  $V_T$  were observed in individuals who used cannabis regularly relative to healthy controls. Taken together with findings from these other studies, our results suggest that repeated cannabis exposure can foster downregulation of  $\text{CB}_1$  receptors, though, importantly, the present study is the first to show this effect in females with CUD. A clear causal link between chronic THC exposure and  $\text{CB}_1$  receptor downregulation has been well established in preclinical research,<sup>46–49</sup> which further supports our conclusion that chronic cannabis use led to  $\text{CB}_1$  downregulation among females with CUD in this study. However, more research is needed to understand how chronic cannabis use and different aspects of cannabis use disorder (e.g., withdrawal) impact  $\text{CB}_1$  receptor availability.

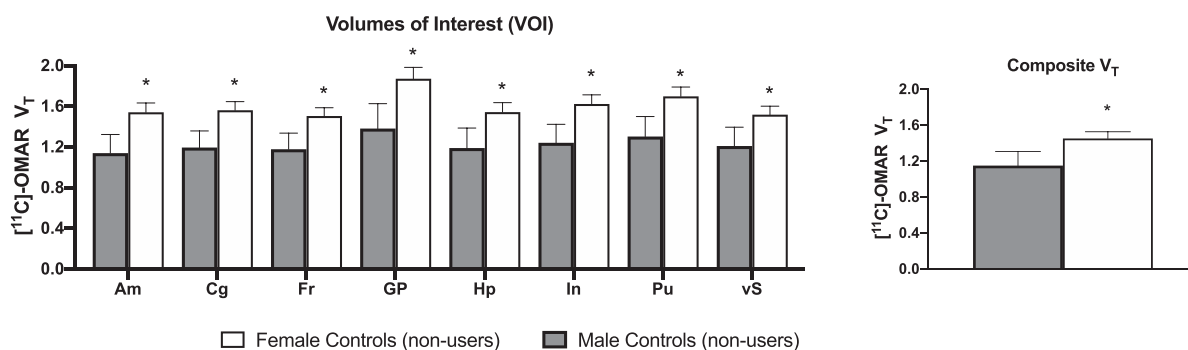
An alternative possible explanation for females with CUD having lower  $V_T$  is that residual THC interfered with radiotracer binding. However, several lines of evidence cast doubt on this possibility. The radiotracer used ( $^{11}\text{C}$ ]OMAR) was developed as an antagonist/inverse agonist, in part, because this would reduce the extent to which  $\text{CB}_1$  agonists (e.g., THC) interfere with its binding. Similar to the present study, D'Souza et al.<sup>15</sup> found that males with CUD had lower  $V_T$  than healthy controls; the authors noted that they performed experiments showing intravenously-administered THC did not interfere with  $^{11}\text{C}$ ]OMAR binding in non-human primates. Similarly, our group verified that acute THC administration (10–40 mg/kg; IP injection) did not displace  $^{11}\text{C}$ ]OMAR in rodents (Wong, unpublished findings). Moreover, in other preclinical research, mice chronically given THC did not have residual THC in their brains one day after THC cessation and the affinity of a  $\text{CB}_1$  antagonist (i.e., [ $^3\text{H}$ ] SR141716) did not differ between the mice given THC vs. those that were given vehicle following THC cessation.<sup>48</sup> Thus, overall, we are confident based on the available data that our results are suggestive



**FIGURE 2** Mean ( $\pm$ SEM) data for heart rate (beats per min, bpm), subjective drug effect ratings (“Drug Effect” and “Like”), and blood concentrations of THC and THC metabolites (11-OH-THC, THCCOOH), over time for female cannabis users ( $N = 14$ ) during the two acute cannabis administration sessions: placebo (i.e., 0-mg THC) and active (i.e., 25-mg THC). Note that blood THC data are only displayed from active sessions



**FIGURE 3** Mean ( $\pm$ SEM)  $V_T$  values for eight volumes of interest (VOIs) and composite  $V_T$ . Asterisks (\*) signify significant differences between females with CUD and female healthy controls who did not use cannabis ( $p < 0.05$ ). On average, females with CUD showed a 16–18% decrease in  $V_T$  in the amygdala, cingulate, hippocampus, and insula compared with controls



**FIGURE 4** Mean ( $\pm$ SEM)  $V_T$  values for eight volumes of interest (VOIs) and composite  $V_T$ . Asterisks (\*) signify significant differences between male and female healthy controls who did not use cannabis ( $p < 0.05$ ). On average, female controls showed a 20–26% increase in  $V_T$  in the eight VOIs and a 21% increase in composite  $V_T$  compared with male controls



of CB<sub>1</sub> downregulation in females with CUD, as opposed to differential radiotracer binding from residual THC in these individuals vs. healthy controls.

Notably, in prior studies of males with CUD,<sup>15,18</sup> CB<sub>1</sub> downregulation reversed with continued cannabis abstinence. For example, in one study,<sup>18</sup> male cannabis users had lower regional V<sub>T</sub> than controls at baseline (i.e., <24 h into a monitored cannabis abstinence period). When scanned again after 13–32 days of abstinence, V<sub>T</sub> had increased significantly relative to baseline. In another study,<sup>15</sup> male cannabis users had lower composite and regional V<sub>T</sub> than controls at baseline, when participants were not intoxicated nor experiencing any symptoms of cannabis withdrawal, and CB<sub>1</sub> receptor downregulation was no longer evident after just 48 h of cannabis abstinence; interestingly, in the present study, V<sub>T</sub> differences were observed in some brain regions between females with CUD and healthy controls 72 h after cannabis abstinence, suggesting the extent of CB<sub>1</sub> downregulation may have been greater or more persistent in the females in this study relative to the males in the study of D'Souza et al.<sup>15</sup> Overall, these collective findings suggest that CB<sub>1</sub> downregulation reflects a state, rather than a trait, condition and may be an explanatory mechanism for the development of aspects of CUD including tolerance, dependence, and craving/withdrawal symptoms during abstinence.

Unlike two prior CB<sub>1</sub>-focused PET studies,<sup>16,18</sup> the vast majority of participants in this study did not use tobacco products. Thus, the lower V<sub>T</sub> observed in females with CUD was not due to tobacco use, which has been associated with lower CB<sub>1</sub> receptor availability in men.<sup>50</sup> Because cannabis and tobacco are often used concurrently<sup>51–53</sup> and users of both substances report more severe withdrawal during abstinence than those who use tobacco or cannabis alone,<sup>54</sup> future PET studies should systematically evaluate the independent and combined effects of nicotine/tobacco and cannabis use on CB<sub>1</sub> availability.

Significant negative correlations were detected between V<sub>T</sub> in the amygdala and hippocampus and Anger/Hostility, a common symptom of cannabis withdrawal,<sup>28</sup> indicating that participants with greater increases in Anger/Hostility during cannabis abstinence had lower V<sub>T</sub> in these regions. Though participants exhibited increases in other withdrawal symptoms and craving during monitored cannabis abstinence, these outcomes were of low magnitude (which is not uncommon in residential research settings) and not correlated with V<sub>T</sub> of specific brain regions, nor composite V<sub>T</sub>. Of the two other studies that examined relations between V<sub>T</sub> and cannabis withdrawal/craving symptoms among males, one found a significant correlation between composite V<sub>T</sub> and withdrawal severity<sup>15</sup> while the other found no associations between V<sub>T</sub> (of various brain regions) and cannabis withdrawal/craving.<sup>18</sup> Additional studies are needed to clarify whether V<sub>T</sub> is related to cannabis withdrawal severity or craving during abstinence observed in an outpatient setting, and whether these relations differ between men and women.

Among healthy controls, females displayed greater V<sub>T</sub> compared with males. These findings are consistent with two prior PET studies,<sup>14,30</sup> but this is the first study to show this effect in males and females matched by age and BMI and with all females

completing PET imaging in their follicular menstrual phase. Contrary to the present study, one PET study<sup>29</sup> showed higher regional V<sub>T</sub> in male versus female non-cannabis users, though importantly, this study did not control for menstrual phase; thus, differences in circulating hormone levels may have contributed to these discrepant findings. There are similar discrepancies across preclinical studies with respect to the directionality of CB<sub>1</sub> receptor availability differences between males and females. There is some preclinical evidence that CB<sub>1</sub>-related sex differences are influenced by hormones and may be brain region-specific. For example, in one study,<sup>55</sup> female rats had higher densities of CB<sub>1</sub> receptors in the amygdala compared with males, but lower CB<sub>1</sub> density in the hypothalamus than males; moreover, this effect was sex steroid-dependent, as an ovariectomy resulted in upregulation of CB<sub>1</sub> receptors in some regions among females.<sup>55</sup>

Also of relevance, the study by Laurikainen et al.<sup>29</sup> used a different CB<sub>1</sub> radiotracer ([<sup>18</sup>F]FMPEP-d2) than the present study and other studies with consistent findings to ours. There are noteworthy differences between [<sup>11</sup>C]OMAR and [<sup>18</sup>F]FMPEP-d2 that may also explain the conflicting results between the present study and Laurikainen et al.<sup>29</sup> First, [<sup>11</sup>C]OMAR is an antagonist/inverse agonist whereas [<sup>18</sup>F]FMPEP-d2 is an inverse agonist. CB<sub>1</sub> receptors exist in either active or inactive states (agonists have a strong preference for binding to active sites whereas antagonists bind to both inactive and active sites similarly).<sup>56</sup> Moreover, it has been posited that CB<sub>1</sub> agonists may temporarily alter the proportion of active versus inactive sites, such that more active sites are available in the presence of agonists.<sup>56</sup> Thus, it is possible binding of inverse agonist radiotracers such as [<sup>18</sup>F]FMPEP-d2 may be impacted by endogenous endocannabinoid agonists. Second, the brain kinetics of [<sup>11</sup>C]OMAR and [<sup>18</sup>F]FMPEP-d2 are distinct and require different types of mathematical modelling. In general, the kinetics of [<sup>11</sup>C]OMAR are faster than [<sup>18</sup>F]FMPEP-d2, allowing for quantification of V<sub>T</sub> in a relatively shorter time (i.e., 90 min). Conversely, [<sup>18</sup>F]FMPEP-d2 kinetics are far slower, with a longer terminal clearance and half-life due to higher CB<sub>1</sub> affinity; thus, [<sup>18</sup>F]FMPEP-d2 requires longer scan times and mathematical modelling to account for the delayed washout time.<sup>57</sup> Ultimately, studies directly comparing V<sub>T</sub> derived from [<sup>11</sup>C]OMAR versus [<sup>18</sup>F]FMPEP-d2 in the same individuals (controlling for hormone levels) are likely needed to fully understand the source of the differences across studies.

Nevertheless, results from the present study suggest that women possess innate differences in CB<sub>1</sub> receptor availability relative to men. These innate differences may be an explanatory mechanism for cannabis-related sex differences observed in prior studies. For example, relative to men, women report stronger ratings for abuse-related subjective effects (e.g., “take again”) after cannabis self-administration<sup>19</sup> and develop CUD at a faster rate following cannabis initiation<sup>25,26</sup>; both of these findings could be influenced by women's innately higher V<sub>T</sub> in regions involved with drug reward (e.g., ventral striatum). Additionally, women exhibit more severe withdrawal symptoms upon abstinence from regular cannabis use than men,<sup>22,23</sup> and women are more susceptible to experiencing adverse acute

effects of cannabis (e.g., acute paranoia and anxiety)<sup>20</sup>; such findings could at least be partially explained by women's innately higher  $V_T$  in regions such as the amygdala.

The strengths of the present study included rigorous control for age and BMI with group matching, limiting tobacco use, and controlling for time since last cannabis use and menstrual cycle phase for PET scans. However, this study had several limitations. First, only females with moderate to severe CUD were included. Thus, although cannabis use and CUD severity was similar among participants, it is unclear whether our findings apply to females who use cannabis less frequently; future studies may also consider including both males and females with CUD to explore whether any study findings differ as a function of sex (e.g., magnitude of  $CB_1$  downregulation). Second, only one PET scan was conducted. Future studies of females with CUD should include PET scans during typical cannabis use and several times during cannabis abstinence to better understand temporal alterations in  $V_T$ . Third, during the cannabis administration sessions, only one active dose of cannabis was administered (25-mg THC). Given that these participants used cannabis daily, they were likely tolerant to the effects of cannabis, particularly at this modest dose. It may be beneficial in future studies of this nature to administer higher doses of THC and/or to include individuals who use cannabis intermittently. Fourth, the relatively small sample size may have limited our statistical power for some analyses, particularly for the correlations between  $V_T$  values and behavioural outcomes. Lastly, as mentioned above, cannabis withdrawal symptoms were measured in an inpatient research setting, which may have influenced responding.

In sum, females with CUD had lower  $CB_1$  receptor availability in various brain regions relative to female healthy controls who did not use cannabis. This suggests that, as has been observed previously in males,<sup>15,18</sup> chronic cannabis use fosters downregulation of  $CB_1$  receptors in females. This study also found that, among non-users of cannabis, females generally displayed higher  $CB_1$  availability than males. Lastly,  $CB_1$  availability (in the amygdala and hippocampus) was negatively correlated with subjective Anger/Hostility ratings during monitored cannabis abstinence, but was not correlated with other withdrawal symptoms or acute cannabis effects. With cannabis legalization continually expanding, it is important to examine the relationship between  $CB_1$  availability and behavioural outcomes among a variety of cannabis-using populations (e.g., infrequent vs. daily users; medicinal vs. "recreational" users; young vs. middle-age vs. older adults with CUD).

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## CONFLICT OF INTEREST

Dr. Spindle has served as a paid consultant for Canopy Health Innovations Inc. Dr. Vandrey has served as a paid consultant for Canopy Health Innovations Inc. and has received honoraria for serving on the scientific advisory boards of FSD Pharma and Present Life Corporation. Dr. Wong has been the PI on studies funded by Roche Neuroscience, LB pharmaceuticals, and Lundbeck. The remaining authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

The study was conceptualized by authors TRS, HW, RV, DFW, and EMW. TRS, HW, and AN conducted data analysis. TRS drafted the manuscript. All authors provided intellectual contributions to the revised manuscript. All authors approved the manuscript for content and approved the final version for publication.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

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

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