Epilepsy & Behavior Reports 12 (2019) 100341

Contents lists available at ScienceDirect

Epilepsy & Behavior Reports

journal homepage: www.elsevier.com/locate/ebcr

A preliminary study of the effects of cannabidiol (CBD) on brain structure in patients with epilepsy

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ARTICLE INFO

Article history:
Received 10 July 2019
Received in revised form 13 September 2019
Accepted 12 October 2019
Available online 9 November 2019

Keywords:
Cannabidiol (CBD)
Treatment-resistant epilepsy (TRE)
Voxel-based morphometry (VBM)
Cortical thickness
Seizure frequency

ABSTRACT

Cannabis use is associated with changes in brain structure and function; its neurotoxic effects are largely attributed to Δ^9-tetrahydrocannabidiol. Whether such effects are present in patients with epilepsy exposed to a highly-purified cannabidiol isolate (CBD; Epidiolex®; Greenwich Biosciences, Inc.) has not been investigated to date. This preliminary study examines whether daily CBD dose of 15–25 mg/kg produces cerebral macrostructure changes and, if present, how they relate to changes in seizure frequency. Twenty-seven patients with treatment-resistant epilepsy were recruited from the University of Alabama at Birmingham CBD Program. Participants provided seizure frequency diaries (SF), completed the Chalfont Seizure Severity Scale (CSSS) and Adverse Events Profile (AEP), and underwent MRI before CBD (baseline) and after achieving a stable CBD dosage (on-CBD). We examined T1-weighted structural images for gray matter volume (GMV) and cortical thickness changes from baseline to on-CBD in 18 participants. Repeated measures t-tests confirmed decreases in SF [$t(17) = 3.08$, $p = 0.0069$], CSSS [$t(17) = 5.77$, $p < 0.001$], and AEP [$t(17) = 3.04$, $p = 0.0074$] between the two time-points. Voxel-level paired samples t-tests did not identify significant changes in GMV or cortical thickness between these two time-points. In conclusion, short-term exposure to highly purified CBD may not affect cortical macrostructure.

1. Introduction

Of the 1.2% of the population that suffers from epilepsy, one-third has treatment-resistant epilepsy (TRE) in which anti-seizure drug (ASD) mono- or poly-therapy does not control seizures [1]. In TRE, the primary tissue insult from chronic, uncontrolled seizures, combined with the secondary effects of failed ASDs, results in on-going insult to brain structure and function [2,3]. Patients with TRE are thus at increased risk for epilepsy-related mortality (e.g., sudden unexpected death in epilepsy; SUDEP), as well as more severe cognitive and neuropsychological impairments [2,3]. While ASDs treat seizures, they do not interrupt or reverse the underlying epileptogenesis [1] which underscores the necessity of finding treatments that interrupt or reverse the pathophysiology that underlies epilepsy. Recent evidence points to chronic neuroinflammation as one of the potential drivers of epileptogenesis [4–8]. Perpetual activation of the neuroinflammatory cascade can lower seizure threshold, resulting in dysfunction of the blood–brain-barrier and chronic neuronal hyperexcitability [4–8]. This notion highlights an under-exploited therapeutic target: the development of treatments that interrupt the neuroinflammatory cascade to provide seizure freedom to patients with TRE.

Cannabis has been used as complementary medicine for a variety of conditions, including epilepsy [9]. In the 1970s–80s, the two most abundant phytocannabinoids, Δ^9-tetrahydrocannabidiol (Δ^9-THC) and cannabidiol (CBD), were chemically identified, isolated, and synthetically manufactured [10]. This allowed empirical investigation of the reported phytocannabinoids' anti-seizure properties. Since then, several animal models have confirmed the anti-seizure properties of Δ^9-THC and CBD, thus renewing interest in their therapeutic potential for humans [11–14]. The most well-understood cannabis actions are attributable to Δ^9-THC, which attenuates seizure frequency in many models; however, its psychotropic and cognitive effects render it an undesirable therapeutic option [11,15]. With the shift in legality surrounding phytocannabinoids, there is an increased interest in cannabidiol (CBD), a potentially superior treatment option to Δ^9-THC due to its

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https://doi.org/10.1016/j.ebr.2019.100341

non-euphoric, neuroprotective, and anti-neuroinflammatory effects [16].

Endocannabinoids' actions at the intersection of the endocannabinoid system (ECS) and immune system drive our interest in CBD's pharmacologic effects. In general, cannabinoid 1 and 2 receptors (CB_1R and CB_2R) are found throughout the central nervous system ($CB_1R > CB_2R$) and on immune cells such as microglia ($CB_1R < CB_2R$) [17,18]. The ECS homeostatically balances excitatory and inhibitory synaptic transmission via CB_1Rs and CB_2Rs—a balance that is not conserved in epilepsy [19]. Due to their expression on immune cells, these receptors—notably CB_2Rs—also play an important role in neuroinflammation [18]. Studies have revealed the dual-nature of endocannabinoids with concentration and context driving either their pro- or anti-inflammatory effects [1,17,20,21]. This duality is evident in their ability to activate and recruit microglia, affect apoptosis, inhibit cell proliferation, and induce regulatory T cells [4,17,19–21]. Endocannabinoids' immunosuppressive effects are best demonstrated by their inhibition of CB_2R-mediated release TNF-α, IL-6, and IL-8, major pro-neuroinflammatory cytokines implicated in epileptogenesis [1,18,20]. In this framework, the autoimmune nature of epilepsy underscores the importance of harnessing endocannabinoids' immunomodulating effects and their ability to modulate the ECS. CBD's anti-epileptic mechanism is not yet fully elucidated, but evidence suggests it decreases neuronal hyperexcitability by ECS-dependent inhibition of excitatory glutamatergic neurotransmission; CBD's anti-inflammatory effects in central and peripheral tissues have not been clearly delineated [11,12,21,22].

Studies have demonstrated significant decreases in seizure frequency and severity following CBD administration [23–25]. It has also been shown that CBD may alter the effects of the co-administered ASDs [26,27]. Structural and functional neuroimaging studies are necessary to better understand CBD's impact on the central nervous system, especially in the context of the developing brain [28]. Our gaps in knowledge about CBD's mechanism of action, coupled with our understanding of cannabis' negative effects, make it increasingly important to further delineate how a CBD isolate differs from cannabis as a whole.

Numerous studies examined the effects of cannabis on brain morphology and function [29]. Despite great variability and inconsistent methodology, structural neuroimaging studies' results converge on abnormalities in CB_1R-dense brain regions [29,30]. For example, Battistella et al. showed that regular cannabis use reduces gray matter volume (GMV) in the temporal, insular, and orbitofrontal cortices—regions rich in CB_1Rs and frequently involved in seizure initiation and generation [31]. Further, studies of cannabis users have demonstrated GMV reductions and cortical thinning in the hippocampus, amygdala, and other subcortical structures, with volume reductions increasing as a function of heavier use [32]. These structural abnormalities have been linked with corresponding functional deficits—for example, hippocampal GMV decreases are associated with decreased working memory [32]. It is not clear whether such effects are attributable to its individual constituents e.g., Δ^9-THC or CBD, or to a combination of all cannabis plant constituents [31]. There are insufficient neuroimaging data on participants administered purified CBD and imaging has not examined whether neuroanatomical alternations may result from such administration to patients with TRE [28].

Previous investigations of Δ^9-THC-induced neuromorphometric changes have used two key techniques that allow quantifying changes in cerebral structure from different but complementary sources [33]. Voxel-based morphometry (VBM) permits a general quantification of GMV change while surfaced-based morphometry pinpoints changes in columnar architecture. By treating the brain as a continuous sheet, surface-based morphometry allows quantifying cortical thickness, as well as other surface-based measures such as sulcus depth, gyrification index, and cortical complexity. Together, these techniques yield a comprehensive account of cerebral macrostructure. In this preliminary study, we use these techniques to investigate the effects of CBD administration on GMV and cortical thickness in TRE. Our aim was to prospectively investigate whether short-term exposure to CBD (Epidiolex®) produced any neuromorphometric changes in participants with TRE. The study also aimed to explore whether changes in GMV and cortical thickness, if present, corresponded to changes in seizure frequency, severity, and adverse events. Based on current understanding of CBD's actions, we hypothesized that there would be no short-term structural brain resulting from continuous exposure to pharmaceutical grade CBD.

2. Methods

2.1. Participants

Following the passage of "Carly's Law," the University of Alabama at Birmingham (UAB) Cannabidiol Program was funded to investigate the safety and efficacy of CBD in TRE. The present study recruited 27 MR-compatible participants from the parent study of 169 participants. Of the recruited participants, one died due to SUDEP; five were excluded because of lack of follow-up data and/or movement artifacts; additional participants ($N = 3$) were not included due to surgical removal of large cortical areas that made their MRI scans unamenable to neuromorphometric analyses. Included participants ($N = 18$; 12 females) ranged from 16 to 73 years of age. The UAB Institutional Review Board approved all study procedures after appropriate Food and Drug Administration and Drug Enforcement Agency approvals and licenses were obtained. The GW Research Ltd. provided highly purified CBD extract (Epidiolex®). All participants were screened for MR compatibility. Written informed consent was obtained from all participants before initiating the protocol.

Recruitment methods, exclusionary/inclusionary criteria, and data collection procedures have been previously published [25]. In brief, healthcare providers referred patients based on criteria available at www.uab.edu/cbd. Of importance is that participants in this study had to have no contraindications to MRI/fMRI at 3 T and had to be able to comply with all neuroimaging study procedures. Participants' doses of ASDs needed to be stable for at least one month prior to enrollment. However, changes to ASD dosing were permissible in the event of suspected drug interactions or side effects.

2.2. Study visits and data collection procedures

Participants were seen for data collection at UAB at two time points: prior to CBD initiation (baseline condition; pre-CBD) and at ≥10 weeks following CBD initiation (on-CBD condition). Each visit consisted of a clinical component (conducted at the weekly research clinic) and a neuroimaging component. A pharmaceutical formulation of highly purified CBD in oral solution (100 mg/mL; Epidiolex® in the U.S.; GW Research Ltd., Cambridge, United Kingdom) was added to each participant's baseline ASD regimen. CBD was initiated at a daily dose of 5 mg/kg, with biweekly titration based on response and tolerability. Doses were taken approximately 12 h apart and combined with other ASDs. At the time of on-CBD imaging, participants maintained a stable CBD dosage of 15–25 mg/kg/day.

2.3. Measures

At each clinic visit (pre- and on-CBD), participants provided seizure diaries, and completed the Chalfont Seizure Severity Scale (CSSS) and Adverse Events Profile (AEP) inventory. Seizure frequency (SF) was calculated from each participant's documented detailed seizure diary, which was kept from 12 weeks prior to CBD initiation to the study's conclusion [34]. CSSS scores served as a standardized measure of seizure severity [35]. The AEP inventory assessed adverse events and other unwanted effects resulting from the CBD intervention in conjunction with other co-administered ASDs [36].

As in our previous reports, baseline evaluations for SF, CSSS, and AEP were based on the 12 weeks preceding CBD initiation [25,26]. For this study, baseline SF was calculated as an average per 28-day period across the preceding 12 weeks. On-CBD evaluations of SF, CSSS, and AEP were based on the time period since CBD initiation. Baseline and on-CBD visits were spaced on average 12.8 ± 4.1 (range 10–24) weeks apart. The difference in SF, CSSS scores, and AEP scores from baseline to on-CBD was used as a measure of CBD's efficacy.

2.4. Imaging protocols

Participants underwent MRI scanning at baseline and on-CBD. Due to scanner upgrade while the study was ongoing, images were acquired on two scanners. For all participants included in data analysis, baseline and on-CBD scans were acquired on the same scanner. Participants were placed in the supine position with earbuds to attenuate scanner noise and cushions to minimize movement. Ten participants received both MRIs on a Siemens Magnetom Allegra 3 T scanner with a circular polarization head coil using a T1-weighted three-dimensional sagittal magnetization-prepared sequence: 192 slices, repetition time (TR) = 2300 ms, echo time (TE) = 2.17 ms, flip angle (FA) = 9°, field of view (FOV) = 256 mm × 256 mm × 192 mm, matrix size = 256 × 256, slice thickness = 1 mm, and voxel size = 1 mm × 1 mm × 1 mm. Images on a Siemens Magnetom Prisma 3 T scanner were acquired with a 20-channel head coil using a T1-weighted three-dimensional sagittal magnetization-prepared sequence with the following parameters: 192 slices, TR = 2300 ms, TE = 3.37 ms, FA = 9°, FOV = 256 mm × 256 mm × 192 mm, matrix size = 256 × 256, slice thickness = 1 mm, and voxel size = 1 mm × 1 mm × 1 mm.

2.4.1. Voxel-based morphometry

Participants' structural images were pre-processed using the Computational Anatomy Toolbox (CAT12) in Statistical Parametric Mapping (SPM12; http://www.fil.ion.ucl.ac.uk) running in MatLab R2017b (The MathWorks, Inc., Natick, MA, USA). All images were reoriented in MRIcron to the same spatial orientation and point of origin (anterior commissure). Preprocessing included skull-stripping, bias correction, tissue segmentation, spatial normalization, and spatial smoothing [33].

Classic SPM12 segmentation methods included skull-stripping based on tissue probability maps (TPMs), with mutual initial affine registration (based on standard ICBM template) for initial spatial registration and segmentation. Images were bias corrected for magnetic field inhomogeneities. The processing pipeline then followed CAT12 Spatial-Adaptive Maximum A Posterior (AMAP) segmentation with Partial Volume Estimation (PVE) to precisely classify tissues into GM, WM, and cerebrospinal fluid (CSF) [37]. Initial affine preprocessing (APP) bias correction accounted for intensity inhomogeneities, while local adaptive segmentation (LAS) corrected for local intensity variations to improve tissue class estimation. The Spatial-Adaptive Non-Local Means (SANLM) denoising filter was applied after intensity normalization, and the Markov Random Field (MRF) denoising was included in AMAP segmentation.

Tissue segments were spatially normalized (1.5 mm voxel size) into standard Montreal Neurological Institute (MNI) space by the high-dimensional Diffeomorphic Anatomical Registration through the Exponentiated Lie Algebra (DARTEL) approach and Geodesic Shooting implemented in CAT12 [38,39]. For each participant, intensity modulation of normalized tissue segments based on Jacobian determinants accounted for global affine transformations and local warping that create unwanted volume changes during spatial normalization.

Images were quality checked using the "Check Sample Homogeneity" function in the CAT12 toolbox, which generated a correlation matrix between volumes to check the most deviating values. CAT12 image quality reports rated resolution, noise, and bias for each participant scan, which were combined into a weighted image quality rating (IQR). Since IQRs are based on raw data before preprocessing, images were not excluded or included solely on the basis of these values. Rather, IQRs were secondarily assessed when image quality was in question based on visual inspection, preprocessing errors, or deviating values in the correlation matrix. Modulated images were spatially smoothed using the default 8-mm full-width-at-half-maximum (FWHM) Gaussian smoothing kernel.

2.4.2. Cortical thickness estimation

The CAT12 toolbox was utilized for fully automated volume-based cortical thickness analyses. Projection-based thickness (PBT) estimated cortical thickness for the left and right hemispheres in one step (during AMAP segmentation described for VBM pipeline) [40]. For central surface reconstruction, WM distance was estimated and local maxima were projected onto other GM voxels using WM distance that describes neighboring relationships [40]. The central surface was generated at the 50% distance between boundaries of GM/WM and GM/CSF. The pipeline included topology correction, spherical inflation, and spherical registration with an adapted DARTEL algorithm [40–42]. The merged surface data for the right and left hemispheres were then resampled and spatially smoothed using the default 15-mm full-width-at-half-maximum (FWHM) Gaussian smoothing kernel.

2.4.3. Post-processing and data harmonization

Please see "Supplement, Section 1" for detailed methods and rationale. For both, VBM and cortical thickness estimations, standard procedures for data harmonization between scanners were utilized.

2.5. Statistical analyses

2.5.1. Participant characteristics and clinical measures of seizure symptoms

Descriptive statistics were obtained for demographic data in IBM SPSS Version 25.0 for Mac. Descriptive statistics for measures of seizure frequency, seizure severity, and adverse events at baseline and on-CBD were also tabulated. Paired t-tests compared SF, CSSS, and AEP at baseline vs. on-CBD.

2.5.2. Voxel-based morphometry analyses

To assess voxel-level GMV changes from baseline to on-CBD, we performed repeated measures t-tests. CAT12 was used to estimate total intracranial volume (TIV), and to construct the statistical model. SPM was used to estimate the statistical model, check design orthogonality, define contrasts, and visualize results. Absolute threshold masking was set to 0.1 to isolate analyses to GM only. Due to the lack of previous VBM evidence, participants' GMV changes were initially assessed with an exploratory whole-brain approach using uncorrected thresholds of $p < 0.001$. For significant GMV changes found with the uncorrected threshold, we then planned to test the models at $p < 0.05$, corrected for multiple comparisons.

TIV was included as a covariate of no interest to correct for different brain sizes by partitioning out the variance of TIV when evaluating for group differences. TIV also accounted for age- and sex-related differences in total brain volume. We also investigated the effect of adding scanner and seizure frequency as explanatory covariates due to high interest in whether GMV changes—if present—were related to scanner type or changes in seizure frequency. If regional changes in GMV were present, we planned to perform multiple univariate regression analyses to assess whether GMV changes were associated with changes in seizure frequency, seizure duration, or seizure severity. However, such analyses were unnecessary due to the lack of GMV change.

2.5.3. Cortical thickness analyses

Statistical models for analyzing cortical thickness were constructed in CAT12 in a manner that mirrored VBM analyses. The resampled and smoothed files for the merged left and right hemispheres for baseline were compared to the on-CBD condition. TIV was not included as a covariate and threshold masking was not included; however, scanner and

seizure frequency were entered as explanatory covariates. The "Estimate Surface Models" function in CAT12 was used to overlay results on the Freesurfer average surface.

3. Results

3.1. Demographics and study measures

Demographic data on the participants were obtained and tabulated (Table 1). Descriptive statistics for demographic data and SF, CSSS, and AEP at baseline and on-CBD were tabulated (Table 2). Repeated measures t-tests compared measures from pre- to on-CBD (Table 3). The repeated measures samples t-tests revealed significant SF reduction $t(17) = 3.08$, $p = 0.007$, CSSS reduction $t(17) = 5.77$, $p < 0.001$, and AEP reduction $t(16) = 3.08$, $p = 0.007$. AEP scores were not available for one participant.

3.2. Voxel-based morphometry results

In the F-test of the overall model, we used a threshold of $p < 0.05$ with corrections for multiple comparisons (family-wise error or FWE). No suprathreshold clusters were present, indicating no significant changes in GMV from baseline to on-CBD. Directional t-tests were conducted to contrast baseline to on-CBD. Bidirectional cluster-level analyses (baseline vs. on-CBD and on-CBD vs. baseline) with a threshold of $p < 0.001$ (uncorrected) revealed no significant differences. The same results were obtained by changing the significance level for each contrast to $p < 0.05$, corrected for FWE, in removing TIV and SF as covariates, and in adding scanner type as a covariate. Null effect sizes are demonstrated in Fig. 1 using box-and-whisker plots of VBM data for representative ROIs at baseline and on-CBD.

3.3. Cortical thickness results

For the F-test of the overall model thresholded at $p < 0.05$ with corrections for multiple comparisons (family-wise error or FWE), no suprathreshold clusters were present indicating no significant differences in cortical thickness between baseline and on-CBD. We also conducted bidirectional t-tests, which documented lack of significant differences when contrasting these timepoints with a threshold of $p < 0.001$ uncorrected or with a $p < 0.05$ corrected for FWE. In contrasting on-CBD to baseline, there were no significant differences with a $p < 0.001$ uncorrected or a $p < 0.05$ corrected for FWE. Null results were maintained after adding scanner and seizure frequency as explanatory covariates.

Table 1
Participant baseline characteristics.

Pt	Epilepsy diagnosis	Age (yrs)	Sex	Onset age (yrs)	Duration (yrs)	#Failed ASDs	Weeks b/w scans
1	TLE	47	M	29	18	6	10
2	Peritumoral	62	F	31	31	14	10
3	TLE	38	M	13	25	4	10
4	TLE	21	F	2	19	4	10
5	FLE	33	M	5	28	5	12
6	Unspecified	24	F	22	2	9	10
7	Right TLE	24	M	2	22	4	14
8	SGE	20	F	2	18	12	14
9	Unspecified	54	F	7	47	7	10
10	FLE	22	F	14	8	4	10
11	TLE	24	F	16	9	5	10
12	FLE	35	F	33	3	4	18
13	Multifocal	28	M	5	23	5	14
14	Unspecified	48	F	8	40	15	16
15	Bi-temporal	34	F	29	3	4	24
16	TLE	73	M	63	10	5	19
17	TLE	55	F	50	5	6	10
18	Unspecified	16	F	13	3	6	10

Abbreviations: TLE = temporal lobe epilepsy; FLE = frontal lobe epilepsy; SGE = symptomatic generalized epilepsy.

Table 2
Descriptive statistics on baseline seizure measures for study participants.

	Males	Females	Combined
N	6	12	18
Age at enrollment (years)	40.5 ± 17.8	34.6 ± 16.1	35.6 ± 16.4
Age at seizure onset (years)	19.5 ± 23.5	18.9 ± 14.5	19.1 ± 17.3
Epilepsy duration (years)	21.0 ± 6.3	15.7 ± 15.7	17.4 ± 13.3
Mean seizure frequency (SF) at baseline	34.5 ± 65.5	26.7 ± 20.7	29.3 ± 39.4
Chalfont Seizure Severity Scale (CSSS) at baseline	56.7 ± 33.3	77.4 ± 47.9	70.5 ± 43.8
Adverse Events Profile (AEP) at baseline	32.3 ± 7.9	49.7 ± 12.1	43.9 ± 13.6

Mean (standard deviation) age at enrollment, age at seizure onset, epilepsy duration, SF, CSSS, in all study participants (N = 18). Mean AEP scores missing for 1 participant (N = 17). SF, CSSS, and AEP scores are based on measures described in the Methods section. Baseline evaluations of SF, CSSS, and AEP were based on the 12 weeks preceding CBD initiation.

4. Discussion

4.1. Main findings

As hypothesized, the present study did not find significant GMV or cortical thickness changes in participants following 10–24 weeks of CBD administration. We have previously demonstrated CBD's safety and efficacy in patients with TRE, specifically in reducing seizure frequency and severity [25]. Despite CBD's positive action at molecular targets to reduce seizure frequency and severity, the lack of structural brain changes further supports the notion that CBD is safe, at least in the short/intermediate term, for TRE patients [15]. These preliminary results add to the growing body of literature on CBD's safety and efficacy, but must be considered in the context of previous findings, CBD's mechanism of action, and study limitations.

Cannabinoids alter brain function; characterizing specific cannabinoids' effects on brain structure, function, and connectivity was not possible until the chemical isolation of individual phytocannabinoids [43–46]. The pharmacological effects of Δ^9-THC and CBD overlap somewhat, as both target the ECS and, to an extent, the immune system. However, their effects may be different and, at times, opposite [15,30,47]. Past work on Δ^9-THC revealed mixed results, but has consistently demonstrated its negative effects (in conjunction with other cannabis plant constituents) on CB_1R-rich structures in heavy, long-term cannabis users [31,46]. Thus, previous findings on cannabis-induced structural changes necessitate investigating whether CBD mediates neuroplasticity. Thus, a central question driving this study was whether CBD alone negatively affects brain structure in patients with TRE? This question is critical since administering CBD for seizure management necessitates daily, long-term use. One study in TRE patients has already demonstrated CBD-induced changes in functional connectivity of the right insula/MFG accompanied by improved cognitive performance in attentional control [48]. Our findings are a further step in elucidating the answer to the principal question of CBD's impact on brain structure and function. The use of two complementary brain morphometric analytical techniques increases our confidence in the results.

4.2. Limitations

The main concern with this study is the potential for type II statistical error, which stems from a number of methodological and sampling limitations that may confound results and decrease statistical power. One limitation of this study was the use of two different scanner types to acquire participants' structural scans. This may have introduced acquisition-related errors and confounded our ability to reliably attribute findings to the CBD intervention rather than scanner effects. Both scanners share the same field strength (3 T), but Prisma's upgraded technology outputs

Table 3
Changes in seizure frequency, seizure severity, and adverse events from baseline to on-CBD.

	Baseline	On-CBD	Baseline vs. on-CBD
Seizure frequency (SF)			
Mean SF	29.3 ± 39.4	10.2 ± 16.8	$t(17) = 3.08$, $p = 0.007$
Chalfront Seizure Severity Scale (CSSS)			
Mean CSSS	70.5 ± 43.8	14.3 ± 17.3	$t(17) = 5.77$, $p < 0.001$
Adverse Events Profile (AEP) inventory			
Mean AEP	43.0 ± 13.4	37.9 ± 12.7	$t(16) = 3.08$, $p = 0.007$

Baseline and on-CBD visits were spaced ~12.8 ± 4.1 (range 10–24) weeks apart.
Mean AEP scores missing for 1 participant (N = 17).

higher quality images than the Allegra, with greater signal-to-noise ratio and enhanced resolution. For all participants included in our analysis, data for both conditions were obtained on the same scanner. Further, though 16 participants experienced 10–16 weeks between scans, CBD titration issues resulted in two participants' on-CBD scans being acquired at 19 and 24 weeks from baseline. VBM and cortical thickness results were unchanged when statistical models included scanner as an explanatory covariate. We also completed post-hoc analyses to further investigate region-based scanner effects before and after ComBat data harmonization (see Supplement, Section 1). Given the results of these post-hoc analyses (see Supplement, Section 2), the type of scanner did not significantly confound our findings. Mid-study scanner upgrades are undesirable, yet inevitable in any study with a long enough data acquisition period. The recent upsurge of multi-site neuroimaging studies has prompted investigation of whether MRI-derived measurements are impacted by scanner upgrades, changes in scanner type, or differing field strengths. Based on this work with short-term VBM studies, within-group inter-scanner variability has a negligible effect on volume differences when scanners are of equivalent field strength, made by the same manufacturers, or operate using the same repetition time or TR—as was the case in our study [49–51]. Thus, based on previous work and the results of our post-hoc analyses on scanner effects, we do not believe that the lack of differences between the two time points is related to scanner upgrade.

Second, the sample size was modest (N = 18). However, previous literature suggests that a repeated measures study design of 18 participants should be adequate to address the questions posed by the study [52]. According to one study, 16 to 32 subjects per group delivers sufficient power for a structural MRI study [53]. To date, ~250 VBM studies have included a maximum of 32 subjects, while ~75 studies have included less than 32 subjects total [52]. Our study was also limited by the heterogeneous sample, which included TRE patients with a broad range of epilepsy diagnoses, disease durations, and seizure symptoms. Aspects of this variation are indirectly demonstrated in participants' SF, CSSS, and AEP scores. For example, mean SF (per 28-day period) at baseline was 29.3 with a standard deviation (SD) of 39.4. The max SF score at baseline was 168, while the minimum SF score was 4. The percent change from baseline to on-CBD was also highly variable. Five participants experienced a 100% decrease (0 seizures for on-CBD condition) with others experiencing lesser reductions (e.g., 14.3% reduction or 42.9% increase). This broad range demonstrates the high variability within our sample, as the TRE patients vary greatly and encompass a broad range of seizure types, ages, and years since diagnosis (Table 1). The broad range of ages and epilepsy subtypes prevented us from reliably assessing subtle, regional differences in GMV that often result from such pathologies. Further, the lack of a shared, focal neuropathology meant that parts of the VBM pipeline were less specific for the

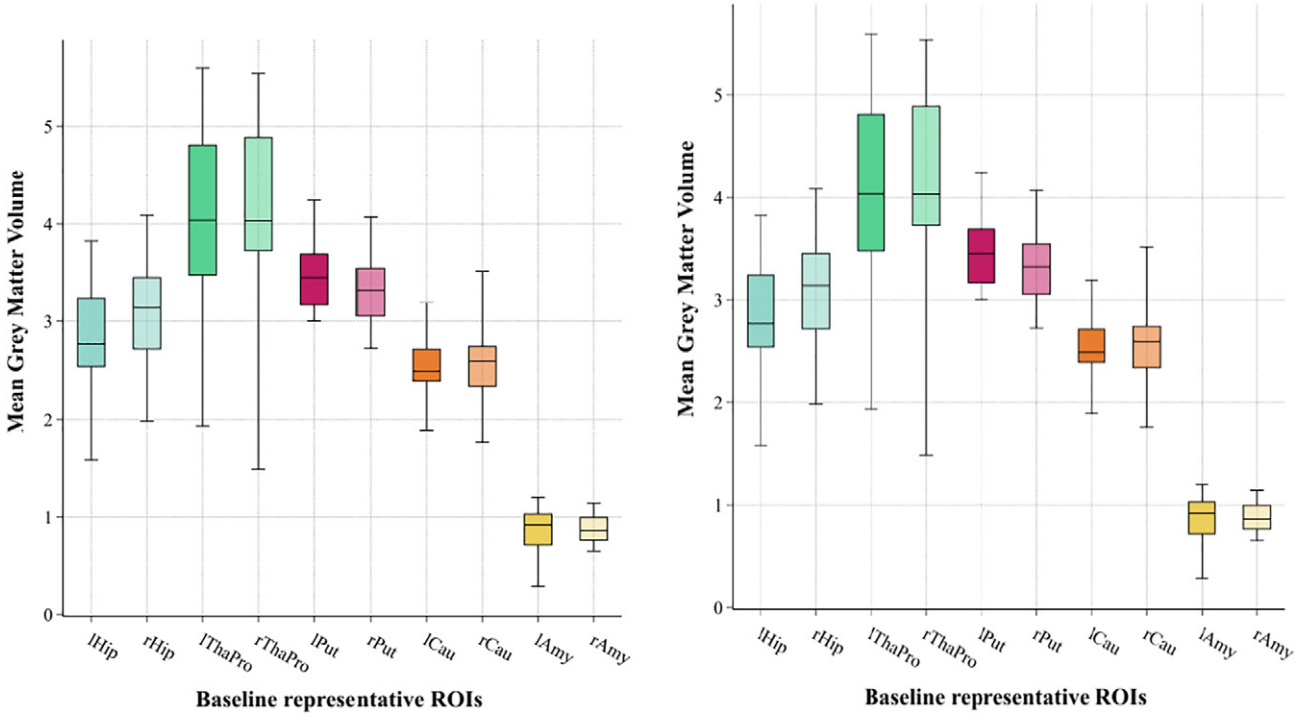

Fig. 1. Null effect sizes demonstrated using VBM data for representative ROIs at baseline and on-CBD. Abbreviations: lHip and rHip: left and right hippocampi; lThaPro and rThaPro: left and right thalami; lPut and rPut: left and right putamina, lCau and rCau: left and right caudates; lAmy and rAmy: left and right amygdalae.

group. For example, the DARTEL toolbox can use a flow-field to generate voxel-by-voxel averages across the group of images to create a study-specific group template. Each participant's scan is then registered to this customized template rather than standard MNI-152 space. The broad range of participants' epilepsy subtypes prevented the use of this group-specific template.

The repeated-measures design of our study and the use of TIV, SF, and scanner type as covariates greatly mitigates our study limitations. The repeated measures design effectively created two groups of 18 participants, in which each participant served as his or her own control. This increased statistical power, and diminished the effects of varying epilepsy diagnoses, slightly different CBD dosages, and use of two different scanners. Future studies would benefit from separately evaluating CBD-induced neuromorphometric changes by epilepsy type/subtype, though such stratification may be difficult when recruiting MR-compatible participants with difficult-to-control epilepsies.

4.3. Implications

VBM and cortical thickness analyses hinge on the idea that microscopic changes may lead to macro-level, MR-detectable differences. However, not all epilepsy participants demonstrate MR-detectable structural abnormalities, which highlights this approach's decreased sensitivity in pinpointing whether a treatment like CBD impacts neuromorphometry. Despite this, previous findings on cannabis-induced structural changes still necessitate investigating CBD-mediated neuroplasticity. Due to the study limitations, short time frame, and lingering questions about CBD's mechanism of action, it is premature to posit that CBD does not change brain structure. This study should be a considered a preliminary step in better articulating CBD's impact on neuromorphometry; our findings provide a necessary foundation for future studies with larger cohorts comprising less variability, more advanced imaging techniques, and longer time between assessments.

5. Conclusion

As hypothesized, our preliminary findings indicate that daily treatment with purified CBD does not change GMV or cortical thickness in patients with TRE. This study further supports CBD's safety and efficacy in a limited sample of patients who were part of a larger dataset. Further longitudinal assessments are critical to ascertain that chronic CBD administration will not change GMV or cortical thickness. If neuroinflammation does indeed underlie epileptogenesis in some patients wtih TRE, functional neuroimaging techniques may be more sensitive for tracking responses to therapy.

Acknowledgments

UAB CBD program is supported by Alabama Department of Commerce ("Carly's Law"). Epidiolex® was provided by GW Research Ltd. (Cambridge, United Kingdom). Dr. Szaflarski is the principal investigator of the adult CBD research program at UAB. UAB CBD studies were registered with www.clinicaltrials.gov under the numbers NCT02695537 and NCT02700412. The authors thank Dr. E. Bebin for referring patients to the study.

Ethical statement

We confirm that any aspect of the work covered in this manuscript that has involved human patients or their protected health information has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. The University of Alabama at Birmingham Institutional Review Board approved all study procedures after appropriate Food and Drug Administration and Drug Enforcement Agency approvals and licenses were obtained. The GW Research Ltd. provided highly purified CBD extract (Epidiolex®). All participants were screened for MR compatibility. Written informed consent was obtained from all participants before initiating the protocol.

Declaration of competing interest

In the last 2 years, Dr. Jerzy Szaflarski has received funding from the National Institutes of Health (NIH), the National Science Foundation (NSF), Charles L. Shor Foundation for Epilepsy Research, EFA, the U.S. Department of Defense, UCB Biosciences, NeuroPace Inc., Sage Therapeutics Inc., Greenwich Biosciences Inc., Serina Therapeutics Inc., and Eisai Inc.; served as a consultant for SAGE Therapeutics Inc., Greenwich Biosciences Inc., NeuroPace, Inc., Upsher-Smith Laboratories, Inc., Medical Association of the State of Alabama, Serina Therapeutics Inc., LivaNova Inc., Lundbeck, and Elite Medical Experts LLC.; serves as an editorial board member for *Epilepsy & Behavior*, *Journal of Epileptology* (associate editor), *Restorative Neurology and Neuroscience* (associate editor), *Journal of Medical Science*, *Epilepsy Currents* (contributing editor), and *Folia Medica Copernicana*. Dr. Jane Allendorfer is a consultant for LivaNova, Inc. and has served as a guest editor for Clinical Therapeutics. Dr. Tyler Gaston has received a consulting fee from Greenwich Biosciences. The remaining authors declare no conflicts of interest.

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebr.2019.100341.

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